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(54) Title: ENHANCED TRANSDERMAL TRANSPORT USING ULTRASOUND

(57) Abstract

Several means for enhancing transdermal transport of drugs and analytes have been developed, including the use of low frequence ultrasound, chemical modifiers of permeability and/or cavitation, iontophoresis and/or electroporation (electric fields), pressure and/or vacuum (physical enhancers), and magnetic force fields. Applications of low-frequency (approximately 20 KHz to 1 MHz) ultrasound enhances transdermal transport of drugs and measurements of the concentration of analytes in body fluids such as blood or lymph. Delivery can be further enhanced or controlled through the use of carriers for the drugs, such as liposomes or microparticles, using a wide range of ultrasound frequency ranges and intensities. The microparticles are preferably small, and may have surfaces with increased hydrophilicity or lipophilicity to further enhance transport.

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ENHANCED TRANSDERMAL TRANSPORT USING ULTRASOUND

Background of the Invention

The present invention is generally in the area of drug delivery, and is particularly improved
5   methodS for transdermal drug delivery and monitoring of analytes.

The United States government has rights in this invention by virtue of National Institutes of Health grant No. GM44884 to Robert Langer and Army
10   Office Grant No. DAAL03-90-G0218 to James C. Weaver.

Transdermal drug delivery (TDD) offers several advantages over traditional delivery methods including injections and oral delivery. When
15   compared to oral delivery, TDD avoids gastrointestinal drug metabolism, reduces first-pass effects, and provides sustained release of drugs for up to seven days, as reported by Elias, In *Percutaneous Absorption: Mechanisms-*
20   *Methodology-Drag Delivery.*, Bronaugh, R. L., Maibach, H. 1. (Ed), pp 1-12, Marcel Dekker, New York, 1989.

Monitoring of analytes using ultrasound has been suggested as an alternative to the use of more
25   invasive procedures. This is particularly attractive for conditions such as diabetes, which requires frequent determination of the blood glucose levels. For example, U.S. Patent No. 5,458,140 to Eppstein, et al., suggests that
30   ultrasound in combination with chemical enhancers is an alternative method for monitoring of blood glucose, although the only examples demonstrate transfer through membranes having pinholes in them.

The word "transdermal" is used herein as a
35   generic term. However, in actuality, transport of drugs occurs only across the epidermis where the drug is absorbed in the blood capillaries. When

compared to injections, TDD eliminates the associated pain and the possibility of infection. Theoretically, the transdermal route of drug administration could be advantageous in the

5   delivery of many therapeutic proteins, because proteins are susceptible to gastrointestinal degradation and exhibit poor gastrointestinal uptake, proteins such as interferons are cleared rapidly from the blood and need to be delivered at

10 a sustained rate in order to maintain their blood concentration at a high value, and transdermal devices are easier to use than injections. Ultrasound has been shown to enhance transdermal transport of low-molecular weight drugs (molecular

15 weight less than 500) across human skin, a phenomenon referred to as sonophoresis (Levy, *J. Clin Invest*. 1989, 83, 2974-2078; Langer, R., In *"Topical Drug Bioavailability, Bioequivalence, and Penetration"*; pp. 91-103, Shah V. P., M.H.I., Eds.

20 (Plenum: New York, 1993); Frideman, R. M., *'Interferons: A Primer'*, Academic Press, New York, 1981)).

U.S. Patent Nos. 4,309,989 to Fahim and 4,767,402 to Kost, et al., disclose various ways in

25 which ultrasound has been used to achieve transdermal drug delivery. Sonophoresis has been shown to enhance transdermal transport of various drugs. Although a variety of ultrasound conditions have been used for sonophoresis, the most commonly

30 used conditions correspond to the therapeutic ultrasound (frequency in the range of 1 MHz - 3 MHz, and intensity in the range of 0 - 2 W/cm^2) (Kost, *In Topical Drug Bioavailability Bioequivalence and Penetration*, pp. 91-103,

35 Maibach, H. I., Shah, V. P. (Ed) Plenum Press, New York, 1993; U.S. Patent No. 4,767,402 to Kost, et al.).

     In spite of these advantages, very few drugs and no proteins or peptides are currently administered transdermally for clinical applications because of the low skin permeability

5   to drugs. Application of therapeutic ultrasound does not induce transdermal transport of high-molecular weight proteins. It is a common observation that the typical enhancement induced by therapeutic ultrasound is less than ten-fold. In

10   many cases, no enhancement of transdermal drug transport has been observed upon ultrasound application. This low permeability is attributed to the stratum corneum (SC), the outermost skin layer which consists of flat, dead cells filled

15   with keratin fibers (keratinocytes) surrounded by lipid bilayers. The highly-ordered structure of the lipid bilayers confers an impermeable character to the SC (Flynn, G. L., In *Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery.*;

20   Bronaugh, R. L., Maibach, H. I. (Ed), pages 27-53, Marcel Dekker, New York, 1989). Examples of *in vivo* monitoring of analytes using ultrasound have not been published.

     A variety of approaches have been suggested to

25   enhance transdermal transport of drugs. These include: i) use of chemicals to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In *"Drug Permeation Enhancement"*; Hsieh,

30   D.S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994; Burnette, R. R. In *Developmental Issues and Research Initiatives*; Hadgraft J., G., R. H., Eds., Marcel Dekker: 1989; pp. 247-288); ii) applications of electric fields to create transient transport

35   pathways [electroporation] (Prausnitz *Proc. Natl. Acad. Sci.USA* 90, 10504-10508 (1993); Walters, K. A., in *Transdermal Drug Delivery: Developmental*

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Issues and Research Initiatives, Ed. Hadgraft J.,
Guy, R.H., Marcel Dekker, 1989) or to increase the
mobility of charged drugs through the skin
[iontophoresis], and iii) application of ultrasound
5   [sonophoresis]. Various approaches including
chemical enhancers [Walters, K.A., in *Transdermal
Drug Delivery: Developmental Issues and Research
Initiatives,* Hadgraft J., Guy, R.H., Marcel Dekker:
New York (1989)], ultrasound [Levy et al., *J. Clin.*
10   *Invest.,* 83: 2074-2078 (1989); Mitragotri et al.,
J. Pharm. Sci, 84:697-706 (1995)] and electrical
enhancement [Prausnitz et al. *Proc. Natl. Acad.
Sci.*USA, 90:10504-10508 (1993); Pliquett et al.,
Pharmaceutical Research, 12:549-555 (1995);
15   Chizmadzhev et al., *Biophysical J.* 68:749-765
(1995); Burnette (1989)] have been suggested to
enhance transdermal drug transport. Chemical
enhancers have been found to increase transdermal
drug transport via several different mechanisms,
20   including increased solubility of the drug in the
donor formulation, increased partitioning into the
SC, fluidization of the lipid bilayers, and
disruption of the intracellular proteins (Kost and
Langer, In Topical Drug Bioavailability,
25   Bioequivalence, and Penetration; Shah and Maibech,
ed. (Plennum, NY 1993) pp. 91-103 (1993)). U.S.
Patent No. 5,445,611 to Eppstein, et al., describes
enhancement of ultrasound using the combination of
chemical enhancers with modulation of the
30   frequency, intensity, and/or phase of the
ultrasound to induce a type of pumping action.
However, the intensity and frequencies used in the
examples are quite high, which generates heat and
decreasing transport over time. Electroporation is
35   believed to work in part by creating transient
pores in the lipid bilayers of the SC (Burnett
(1989)). Iontophoresis provides an electrical

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driving force to move compounds.   Electroporation
involves application of electric field pulses that
create transient aqueous pathways in lipid bilayer
membranes, causing a temporary alteration of skin
5   structure.   While occurrence of aqueous pores may
allow transdermal permeation of neutral molecules
by diffusion, the transport of charged molecules
during pulsing occurs predominantly by
electrophoresis and electroosmosis. In some cases,
10   high strengths of the physico-chemical forces (for
example, electricity, ultrasound) are required to
deliver a given drug dose transdermally. However,
the highest strength of these physico-chemical
forces that can be used is limited by their adverse
15   physiological effects.

        Accordingly, a better selection of ultrasound
parameters is needed to induce a higher enhancement
of transdermal drug transport by sonophoresis.
Moreover, although efficacy to some degree has been
20   observed using ultrasound for transport of other
compounds, the efficiency of transport under
conditions acceptable to patients has not been
achieved.

        It is therefore an object of the present
25   invention to provide a method and means for
enhancing transdermal transport for transdermal
drug delivery and monitoring of analyte.

        It is a further object of the present
invention to provide methods for using ultrasound
30   in combination with other means of enhancement for
drug delivery and collection of analyte in an
efficient, practical manner.

        It is a further object of the present
invention to provide an improved, painless method
35   for obtaining a patient sample for measurement of
analytes in blood or other body fluids.

Summary of the Invention

Several means for enhancing transdermal transport of drugs and analytes have been developed, including the use of low frequency
5   ultrasound, chemical modifiers of permeability and/or cavitation, iontophoresis and/or electroporation (electric fields), pressure and/or vacuum (physical enhancers), and magnetic force fields. Applications of low-frequency
10   (approximately 20 KHz to 1 MHz) ultrasound enhances transdermal transport of drugs and measurements of the concentration of analytes in body fluids such as blood or lymph. In a preferred embodiment, the ultrasound is low frequency ultrasound which
15   induces cavitation, thereby increasing the permeability of the stratum corneum. Delivery can be further enhanced or controlled through the use of carriers for the drugs, such as liposomes or microparticles, using a wide range of ultrasound
20   frequency ranges and intensities. The microparticles are preferably small, and may have surfaces with increased hydrophilicity or lipophilicity to further enhance transport.

Transdermal transport of molecules during
25   sonophoresis (delivery or extraction) can be further enhanced by providing chemical enhancers which increase the solubility of the compound to be transported and/or lipid bilayer solubility, or additional driving forces for transport, such as,
30   mechanical force fields, magnetic fields or iontophoresis.

Transdermal transport of molecules during sonophoresis (delivery or extraction) can be further enhanced by the application of an electric
35   field, for example, by iontophoresis or electroporation. Still further enhancement can be obtained using a combination of chemical enhancers

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and/or magnetic field with the electric field and
ultrasound.

Examples using low frequency ultrasound
demonstrate *in vitro* and *in vivo* administration of
5   insulin (molecular weight 6,000 D), and *in vitro*
administration of gamma interferon (molecular
weight 17,000 D), and erythropoeitin (molecular
weight 48,000 D). Examples using low frequency
ultrasound also demonstrate measurement of blood
10   glucose *in vitro* and *in vivo*. Additional examples
compare the effects and mechanisms of (i) a series
of chemical enhancers, and (ii) the combination of
these enhancers and therapeutic ultrasound (1 MHz,
1.4 W/cm2) on transdermal drug transport.
15   Initial/comprehensive experiments were performed
with a model drug, corticosterone, and a series of
chemical enhancer formulations, including
polyethylene glycol 200 dilaurate (PEG), isopropyl
myristate (IM), glycerol trioleate (GT), ethanol/pH
20   7.4 phosphate buffered saline in a one-to-one ratio
(50% ethanol), 50% ethanol saturated with linoleic
acid (LA/ethanol), and phosphate buffered saline
(PBS). Examples using two model compounds, calcein
and sulphorhodamine, demonstrate that transdermal
25   transport enhancement induced by simultaneous
application of ultrasound and electric pulses is
higher than that due to electric pulses or
ultrasound alone. Application of ultrasound
reduces the threshold voltage required for the
30   onset of calcein and sulphorhodamine transport in
the presence of electric fields.

Brief Description of the Drawings
Figure 1a is a graph of the amount of insulin
transported across human skin (in *vitro*) in the
35   presence of ultrasound (20 KHz, 100 msec pulses
applied every second) at various intensities (■ -

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12.5 mW/cm², ◆ - 62.5 mW/cm², ● - 125 mW/cm², and ▲
- 225 mW/cm²)'. (n=3-4, error bars indicate SD
(Standard Deviation))

Figure 1b is a graph of the variation of the
transdermal insulin permeability (in vitro) with
ultrasound intensity (20 KHz, 100 msec pulses
applied every second). (n=3-4, error bars indicate
SD.) Note that the skin is impermeable to insulin
at an ultrasound intensity =0.

Figure 2 is a graph of glucose concentration
in the donor compartment (measured as percent of
received glucose) over time (minutes) in an in
vitro system.

Figure 3 is a graph of glucose concentration
in the donor compartment (mg/dl) after ten minutes
as a function of glucose concentration in the
receiver compartment (mg/dl).

Figure 4 is a graph of solubility (mg/ml) for
corticosterone, dexamethasone, and testosterone in
PBS (dark bar), 50% ethanol (hatched \\\) and
linoleic acid in 50% ethanol (striped ||||).

Figure 5 is graph of the permeability
enhancement for testosterone (288.4 Da),
corticosterone (346.5 Da), dexamethasone (392.5 Da)
in combination with linoleic acid (dark bars) and
ultrasound in combination with linoleic acid
enhancement (\\\).

Figure 6a is a graph of enhancement (log
scale) versus molecular weight (Da). Figure 6b is
a graph of permeability enhancement versus
molecular weight (Da).

Figure 7a is a graph of fraction of
corticosterone transported (%) versus time (hours).
Figure 7b is a graph of the fraction of
corticosterone transported (%) versus time (hours).

Figure 8 is a graph of the fraction of
corticosterone transported (%) versus time (hours).

Figure 9 is a graph of the amount of calcein transported in one hour (fraction of the amount in the donor x 10^5) for sonophoresis alone, iontophoresis alone, and sonophoresis in

5   combination with iontophoresis.

Figure 10 is a graph of the glucose concentration in the donor (fraction of receiver concentration).

Figure 11a is a graph of sulforhodamine

10   flux/μg/(cm^2h) over time (seconds) for electroporation of sulforhodamine, followed by application of electroporation in combination with ultrasound. After 400 sec of passive diffusion, pulsed ultrasound (1 MHz, 20% duty cycle, 2.5 - 2.9

15   W/cm^2) was turned on for 2750 sec. The ultrasound was turned off at 2750 sec. High voltage pulsing was turned on at 6900 sec for 1 hour (10,500 sec end of electroporation pulsing). Ultrasound (1 MHz, 0.8 cm^2 20% duty cycle, 2.5 - 2.9 W/cm^2) was

20   turned on again at 14,310 sec, electroporation was turned on again at 15,200 sec while the pulsed ultrasound was on. At 16,440 sec the ultrasound wave was changed from pulsed to continuous while the electroporation continued.

25   Figure 11b is a graph of the time variation of calcein flux in the presence of electric fields alone (X) and during simultaneous application of ultrasound and electric field (O) (1 MHz, 1.4 W/cm^2, continuous application, and electric field, 100 V

30   across the skin, exponentially decaying pulse with a time constant (τ) of 1 millisecond, one pulse applied every minute). Ultrasound was ON all the time (O). Electric voltage was turned ON at time 0 and was turned OFF at 1 hour in both the case (O as

35   well as X). Presented as means and S.D. of at least three repetitions.

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Figure 11c. Time variation of sulphorhodamine flux in the presence of electric field alone (X) and during simultaneous application of ultrasound and electric field (O) (1 MHz, 1.4 W/cm^2, continuous
5 application, and electric field, 100 V across the skin, exponentially decaying pulse with a time constant (τ) of 1 millisecond, one pulse applied every minute). Ultrasound was ON all the time (O). Electric voltage was turned ON at time O and was
10 turned OFF at 1 hour in both the case (O as well as X). Presented as means and S.D. of at least three repetitions.

Figure 12a and 12b are graphs of calcein and sulphorhodamine flux over time (hours),
15 respectively. Skin samples were exposed continuously to electroporation (electric field (750 V across the chamber, equivalent to approximately 210-230 volts across the skin, exponentially decaying pulse with a time constant
20 (τ) of 1 millisecond, one pulse applied every minute) and continuous ultrasound (1 MHz, 0.8 cm^2, 2 W/cm^2) (o) and controls (x) where the skin was exposed to electric fields alone.

Figure 13 is a graph of the variation of the
25 transdermal sulphorhodamine flux with the applied electric field (100 V across the skin, exponentially decaying pulse with a time constant (τ) of 1 millisecond, one pulse applied every minute) in the presence (O) and absence (X) of
30 ultrasound. Presented as means and S.D. of at least three repetitions.

Figure 14 is a graph showing the variation of the normalized transdermal calcein and sulphorhodamine flux under a variety of conditions.
35 A- in the presence of electric field alone, B- in the presence of ultrasound and electric field, C- in the presence of ultrasound alone, D- in the

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absence of ultrasound and electric field. The
transdermal calcein and sulphorhodamine fluxes have
been normalized by the corresponding fluxes prior
to application of ultrasound, that is, at the end
5   of 0.5 hours. This was done to assist comparison
of the relative charges in transdermal flux under
different conditions.

Detailed Description of the Invention
Sonophoresis:
10   As used herein, sonophoresis is the
application of ultrasound to the skin, alone or in
combination with chemical enhancers, iontophoresis,
electroporation, magnetic force fields, mechanical
pressure fields or electrical fields, to facilitate
15   transport of a compound through the skin. In one
embodiment, a drug, alone or in combination with a
carrier, penetration enhancer, lubricant, or other
pharmaceutically acceptable agent for application
to the skin, is applied to the skin. In another
20   embodiment, the compound is an analyte such as
glucose which is present in a body fluid and
extracted by application of the ultrasound, alone
or in combination with other forces and/or chemical
enhancers.
25   Ultrasound is defined as sound at a frequency
of between 20 kHz and 10 MHz, with intensities of
between greater than 0 and 3 W/cm^2. Ultrasound is
preferably administered at frequencies of less than
or equal to about 2.5 MHz to induce cavitation of
30   the skin to enhance transport. Exposures are
typically for between 1 and 100 minutes, but may be
shorter and/or pulsed.
As used herein, "low frequency" sonophoresis
is ultrasound at a frequency that is less than 1
35   MHz, more typically in the range of 20 to 40 KHz,
which can be applied continuously or in pulses, for

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example, 100 msec pulses every second, at
intensities in the range of between zero and 1
W/cm², more typically between 12.5 mW/cm² and 225
mW/cm². It should be understood that although the
5   normal range of ultrasound begins at 20 kHz, one
could achieve comparable results by varying the
frequency to slightly more or less than 20 kHz.

Many ultrasound devices are available
commercially which can be used in the method
10   described herein. For example, the ultrasonic
devices used by dentists to clean teeth have a
frequency of between about 25 and 40 KHz.
Commercially available portable ultrasound tooth-
brushes make use of a small sonicator contained
15   within the tooth-brush (Sonex International
Corporation). This sonicator is portable and
operates on rechargeable batteries. Small pocket-
size sonicators carried by patients and used to
"inject" drugs whenever required could be readily
20   adapted from these devices. In addition, these
devices could be combined with sensors that can
monitor drug concentrations in the blood to
formulate a self-controlled drug (insulin, for
example) delivery method that can decrease the
25   attention required by the patient.

Devices typically used for therapeutic or
diagnostic ultrasound operate at a frequency of
between 1.6 and 10 MHz. These devices can also be
modified for use at lower frequencies. The devices
30   may optionally include a reservoir for an
ultrasound gel, which will typically have an
acoustic impedance like water, or a reservoir for
collecting analyte.

Although principally described herein as the
35   combination of ultrasound with an electrical field,
chemical enhancers and physical enhancers can also
be used in combination with ultrasound. Physical

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enhancers, as used herein, in addition to
iontophoresis and electroporation, include magnetic
fields and mechanical pressure or vacuum.
Ultrasound is used to permeabilize the skin

5   followed by the application of various force fields
to provide additional driving force for transdermal
transport of molecules.

Chemical Enhancers.

Lipid Bilayer Disrupting Agents.

10   Chemical enhancers have been found to increase
drug transport by different mechanisms. Chemicals
which enhance permeability through lipids are known
and commercially available. For example, ethanol
has been found to increase the solubility of drugs

15   up to 10,000-fold and yield a 140-fold flux
increase of estradiol, while unsaturated fatty
acids have been shown to increase the fluidity of
lipid bilayers (Bronaugh and Maibach, editors
(Marcel Dekker 1989) pp. 1-12). Examples of fatty

20   acids which disrupt lipid bilayer include linoleic
acid, capric acid, lauric acid, and neodecanoic
acid, which can be in a solvent such as ethanol or
propylene glycol. Evaluation of published
permeation data utilizing lipid bilayer disrupting

25   agents agrees very well with the observation of a
size dependence of permeation enhancement for
lipophilic compounds. The permeation enhancement
of three bilayer disrupting compounds, capric acid,
lauric acid, and neodecanoic acid, in propylene

30   glycol has been reported by Aungst, et al. *Pharm.
Res.* 7, 712-718 (1990). They examined the
permeability of four lipophilic compounds, benzoic
acid (122 Da), testosterone (288 Da), naloxone (328
Da), and indomethacin (359 Da) through human skin.

35   The permeability enhancement of each enhancer for
each drug was calculated according to $\epsilon_{c/pg}=P_{e/pg}/P_{pg}$,
where $P_{e/pg}$ is the drug permeability from the

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enhancer/propylene glycol formulation and P_{pg} is the
permeability from propylene glycol alone.

The primary mechanism by which unsaturated fatty
acids, such as linoleic acid, are thought to
5   enhance skin permeabilities is by disordering the
intercellular lipid domain. For example, detailed
structural studies of unsaturated fatty acids, such
as oleic acid, have been performed utilizing
differential scanning calorimetry (Barry J.
10   Controlled Release 6, 85-97 (1987)) and infrared
spectroscopy (Ongpipattanankul, et al., Pharm. Res.
8, 350-354 (1991); Mark, et al., J. Control. Rel.
12, 67-75 (1990)). Oleic acid was found to
disorder the highly ordered SC lipid bilayers, and
15   to possibly form a separate, oil-like phase in the
intercellular domain. SC lipid bilayers disordered
by unsaturated fatty acids or other bilayer
disrupters may be similar in nature to fluid phase
lipid bilayers.
20   A separated oil phase should have properties
similar to a bulk oil phase. Much is known about
transport in fluid bilayers and bulk oil phases.
Specifically, diffusion coefficients in fluid
phase, for example, dimyristoylphosphatidylcholine
25   (DMPC) bilayers Clegg and Vaz In "Progress in
Protein-Lipid Interactions" Watts, ed. (Elsevier,
NY 1985) 173-229; Tocanne, et al., FEB 257, 10-16
(1989) and in bulk oil phase Perry, et al.,
"Perry's Chemical Engineering Handbook" (McGraw-
30   Hill, NY 1984) are greater than those in the SC,
and more importantly, they exhibit size
dependencies which are considerably weaker than
that of SC transport Kasting, et al., In:
"Prodrugs: Topical and Ocular Delivery" Sloan, ed.
35   (Marcel Dekker, NY 1992) 117-161; Potts and Guy,
Pharm. Res. 9, 663-339 (1992); Willschut, et al.,
Chemosphere 30, 1275-1296 (1995). As a result, the

diffusion coefficient of a given solute will be
greater in a fluid bilayer, such as DMPC, or a bulk
oil phase than in the SC. Due to the strong size
dependence of SC transport, diffusion in SC lipids

5   is considerably slower for larger compounds, while
transport in fluid DMPC bilayers and bulk oil
phases is only moderately lower for larger
compounds. The difference between the diffusion
coefficient in the SC and those in fluid DMPC

10   bilayers or bulk oil phases will be greater for
larger solutes, and less for smaller compounds.
Therefore, the enhancement ability of a bilayer
disordering compound which can transform the SC
lipids bilayers into a fluid bilayer phase or add a

15   separate bulk oil phase should exhibit a size
dependence, with smaller permeability enhancements
for small compounds and larger enhancements for
larger compounds.

A comprehensive list of lipid bilayer disrupting

20   agents is described in European Patent Application
43,738 (1982), which is incorporated herein by
reference. Exemplary of these compounds are those
represented by the formula:

R- X,

25   wherein R is a straight-chain alkyl of about 7
to 16 carbon atoms, a non-terminal alkenyl of about
7 to 22 carbon atoms, or a branched-chain alkyl of
from about 13 to 22 carbon atoms, and X is $-OH$,
$-COOCH_3$, $-COOC_2H_5$, $-OCOCH_3$, $-SOCH_3$, $-P(CH_3)_2O$,

30   $COOC_2H_4OC_2H_4OH$, $-COOCH(CHOH)_4CH_2OH$, $-COOCH_2CHOHCH_3$,
$COOCH_2CH(OR'')CH_2OR''$, $-(OCH_2CH_2)_mOH$, $-COOR'$, or $-CONR'_2$
where R' is $-H$, $-CH_3$, $-C_2H_5$, $-C_2H_7$ or $-C_2H_4OH$; R'' is
$-H$, or a non-terminal alkenyl of about 7 to 22
carbon atoms; and m is 2-6; provided that when R''

35   is an alkenyl and X is $-OH$ or $-COOH$, at least one
double bond is in the cis-configuration.

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Solubility Enhancers

Suitable solvents include water; diols, such
as propylene glycol and glycerol; mono-alcohols,
such as ethanol, propanol, and higher alcohols;
5   DMSO; dimethylformamide; N,N-dimethylacetamide; 2-
pyrrolidone; N-(2-hydroxyethyl) pyrrolidone, N-
methylpyrrolidone, 1-dodecylazacycloheptan-2-one
and other n-substituted-alkyl-azacycloalkyl-2-ones
and other n-substituted-alkyl-azacycloalkyl-2-ones
10 (azones).

U.S. Patent No. 4,537,776 to Cooper contains a
summary of prior art and background information
detailing the use of certain binary systems for
permeant enhancement. European Patent Application
15 43,738, also describes the use of selected diols as
solvents along with a broad category of cell-
envelope disordering compounds for delivery of
lipophilic pharmacologically-active compounds. A
binary system for enhancing metaclopramide
20 penetration is disclosed in UK Patent Application
GB 2,153,223 A, consisting of a monovalent alcohol
ester of a C8-32 aliphatic monocarboxylic acid
(unsaturated and/or branched if C18-32) or a C6-24
aliphatic monoalcohol (unsaturated and/or branched
25 if C14-24) and an N-cyclic compound such as 2-
pyrrolidone or N-methylpyrrolidone.
Combinations of enhancers consisting of diethylene
glycol monoethyl or monomethyl ether with propylene
glycol monolaurate and methyl laurate are disclosed
30 in U.S. Patent No. 4,973,468 for enhancing the
transdermal delivery of steroids such as
progestogens and estrogens. A dual enhancer
consisting of glycerol monolaurate and ethanol for
the transdermal delivery of drugs is described in
35 U.S. Patent No. 4,820,720. U.S. Patent No.
5,006,342 lists numerous enhancers for transdermal
drug administration consisting of fatty acid esters

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or fatty alcohol ethers of C_2 to C_4 alkanediols, where each fatty acid/alcohol portion of the ester/ether is of about 8 to 22 carbon atoms. U.S. Patent No. 4,863,970 discloses penetration-

5   enhancing compositions for topical application including an active permeant contained in a penetration-enhancing vehicle containing specified amounts of one or more cell-envelope disordering compounds such as oleic acid, oleyl alcohol, and

10   glycerol esters of oleic acid; a C_2 or C_3 alkanol and an inert diluent such as water.

Other chemical enhancers, not necessarily associated with binary systems, include dimethylsulfoxide (DMSO) or aqueous solutions of

15   DMSO such as those described in U.S. Patent No. 3,551,554 to Herschler; U.S. Patent No. 3,711,602 to Herschler; and U.S. Patent No. 3,711,606 to Herschler, and the azones (n-substituted-alkyl-azacycloalkyl-2-ones) such as noted in U.S. Patent

20   No. 4,557,943 to Cooper.
Some chemical enhancer systems may possess negative side effects such as toxicity and skin irritations. U.S. Patent No. 4,855,298 discloses compositions for reducing skin irritation caused by chemical

25   enhancer-containing compositions having skin irritation properties with an amount of glycerin sufficient to provide an anti-irritating effect.

Combinations of Lipid Bilayer Disrupting Agents and Solvents

30   Passive experiments without ultrasound with polyethylene glycol 200 dilaurate (PEG), isopropyl myristate (IM), and glycerol trioleate (GT) result in corticosterone flux enhancement values of only 2, 5, and 0.8, relative to the passive flux from

35   PBS alone. However, 50% ethanol and LA/ethanol significantly increase corticosterone passive fluxes by factors of 46 and 900. These passive

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flux enhancements were due to (1) the increased
corticosterone solubility in the enhancers, and (2)
interactions of linoleic acid with the skin.
Specifically, linoleic acid increased the
5   corticosterone permeability by nearly 20-fold over
that from 50% ethanol alone. Therapeutic
ultrasound (1 MHz, 1.4 W/cm^2) and the chemical
enhancers utilized together produce corticosterone
fluxes from PBS, PEG, IM, and GT that are greater
10   than the passive fluxes from the same enhancers by
factors of between 1.3 and 5.0, indicating that the
beneficial effects of chemical enhancers and
therapeutic ultrasound can be effectively combined.
Ultrasound combined with 50% ethanol produces a 2-
15   fold increase in corticosterone transport above the
passive case, but increase by 14-fold the transport
from LA/Ethanol. The combination of increased
corticosterone solubility in and permeability
enhancement from LA/ethanol and ultrasound yields a
20   flux of 0.16 mg/cm^2/hr, 13,000-fold greater than
that from PBS alone.   The permeability enhancement
resulting from the addition of linoleic acid to 50%
ethanol exhibits a clear size dependence, with the
degree of enhancement increasing with the size of
25   the drug. The degree of permeation enhancement
achieved by adding linoleic acid to 50% ethanol and
applying ultrasound exhibits a similar size
dependence. Ultrasound combined with 50% ethanol
produced a 2-fold increase in corticosterone
30   transport above the passive case, but increased by
14-fold the transport from LA/Ethanol. The
combination of increased corticosterone solubility
in and permeability enhancement from LA/ethanol and
ultrasound yields a flux of 0.16 mg/cm^2/hr, 13,000-
35   fold greater than that from PBS alone. In order to
assess the generality of enhancement ability of
LA/ethanol and ultrasound, further experiments were

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performed with two additional model drugs,
dexamethasone and testosterone. As with
corticosterone, the solubilities in and passive
permeabilities from LA/ethanol were much larger
5   than those from PBS alone for dexamethasone and
testosterone. The sonophoretic permeabilities from
LA/ethanol were also greater for these two drugs
than the passive permeabilities. Moreover, the
permeability enhancements of the three drugs
10   resulting from the addition of linoleic acid to 50%
3thanol exhibited a clear size dependence, with the
degree of enhancement increasing with the size of
the drug. The degree of permeation enhancement
achieved by adding linoleic acid to 50% ethanol and
15   applying ultrasound exhibits a similar size
dependence. These results suggest that linoleic
acid and therapeutic ultrasound, which are both
lipid bilayer disordering agents, shift the
transport of lipophilic molecules from the passive
20   regime to a regime with a very weak size
dependence.

Mechanical Forces.

Mechanical or Osmotic Pressure

The advantages of combining sonophoresis with
25   physical enhancers is not restricted to electrical
current. Effects on transdermal transport may also
be observed between ultrasound and pressure
(mechanical or osmotic) as well as between
ultrasound and magnetic fields since the physical
30   principles underlying the enhancement are believed
to be similar or the same. A pressure gradient can
be used to enhance convection (physical movement of
liquid) across the skin permeabilized by
sonophoresis. This can be particularly useful in
35   transdermal extraction of blood analytes.
Application of pressure, for example, a vacuum or
mechanical pressure, to the skin pretreated by

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sonophoresis can result in transdermal extraction
of interstitial fluid which can be analyzed to
measure concentration of various blood analytes.
Electric Fields (Iontophoresis or Electroporation)

5   Application of ultrasound or electric current
alone has been shown to enhance transdermal drug
transport and blood analyte extraction.
Ultrasound-induced cavitation occurring inside or
outside the skin causes enhanced transport.

10   Application of electric current enhances
transdermal transport by different mechanisms.
First, application of an electric field provides an
additional driving force for the transport of
charged molecules across the skin and second, ionic

15   motion due to application of electric fields may
induce convective flows across the skin, referred
to as electroosmosis. This mechanism is believed
to play a dominant role in transdermal transport of
neutral molecules during iontophoresis.

20   Iontophoresis involves the application of an
electrical current, preferably DC, or AC, at a
current density of greater than zero up to about 1
mA/cm^2.

          Attempts have been made to enhance the skin

25   permeability using electric current to achieve
transdermal extraction of glucose, as reported by
Tamada, et al., *Proceed. Intern. Symp. Control.*
Rel. Bioact. Mater. 22, 129-130 (1995). Although
these attempts have been successful to a certain

30   extent, the amounts of glucose extracted by these
methods are several orders of magnitude lower than
those which could be detected by the currently
existing biosensors. The mechanism of sonophoretic
transdermal glucose extraction is believed to be

35   similar to that of sonophoretic transdermal drug
delivery.

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In view of this, the cumulative effect of
ultrasound and electric field may also be related
to cavitation induced by ultrasound exposure. In
order to test this hypothesis, electric pulses (100
5   V across the skin, 1 ms exponential pulse applied
every minute) and ultrasound (*3 MHz*, 1.5 W/cm^2) were
simultaneously applied to skin, as described below.
It is known that the cavitational effects vary
inversely with ultrasound frequency [Gaertner, W.,
10   Frequency dependence of ultrasonic cavitation, *J.
Acoust. Soc. Am.*, 26:977-80 (1984)]. No
significant cavitational effects have been observed
in fluids at high ultrasound frequencies greater
than 2.5 MHz. As a result, 2.5 MHz is considered a
15   reasonable estimate of the upper frequency limit
for the occurrence of cavitation in fluids at
therapeutic ultrasound intensities. Hence, if
cavitation plays an important role, the synergistic
effect of ultrasound and electric field should be
20   nearly absent when 3 MHz ultrasound is used.
Exposure to ultrasound at 3 MHz (intensity = 1.5
W/cm^2) does not affect transdermal transport by
electric field pulsing. These results indicate
that cavitation may play a major role in the
25   synergistic effect of ultrasound and electric field
pulsing.
The combination of sonophoresis with an
electric field, and optionally, any of these
additional physical mechanisms for enhanced
30   transport provides the following advantages over
sonophoresis or the physical enhancers alone: i)
It allows lowering application times to deliver a
given drug dose or extract a certain amount of
analytes compared to the required times in the
35   presence of ultrasound or one of the other
enhancers alone; ii) It reduces the magnitude of
the required ultrasound intensity and electric

current or pressure to achieve a given transdermal flux compared to that required if, the enhancers were used alone; and iii) It can be used to provide a better control over transdermal transport of molecules compared to that obtained using an enhancer alone.

The combination of electrical field and ultrasound can be applied to any membrane. The membrane can be skin, cell membrane, cell wall and other biological as well as synthetic membranes. The electric fields can be continuous, pulsed, having high as well as low voltage. Application of ultrasound together with the electrical fields results in higher flux compared to the flux observed with electroporation or ultrasound alone. The onset time of transdermal flux during electroporation can also be reduced by simultaneous applications of ultrasound and electroporation. The effect is more pronounced on less-charged molecules which by other enhancing methods are hard to enhance (iontophoresis). The major limitation of electroporation are the high voltages required in order to cause significant effect. By using the combined effects of ultrasound and electroporation, the intensity levels of the electrical fields will be much lower and therefore no or less damage to the membranes will be observed.

Magnetic Fields

Application of magnetic fields to the skin pretreated or in combination with ultrasound may also result in a higher transport of magnetically active species across the skin. For example, polymer microspheres loaded with magnetic particles could be transported across the skin using sonophoresis and magnetic fields.

Drug Delivery and Monitoring of Analytes

Drugs to be Administered.

Drugs to be administered include a variety of
bioactive agents, but are preferably proteins or
5   peptides. Specific examples include insulin,
erythropoietin, and interferon. Other materials,
including nucleic acid molecules such as antisense
and genes encoding therapeutic proteins, synthetic
organic and inorganic molecules including
10 antiinflammatories, antivirals, antifungals,
antibiotics, local anesthetics, and saccharides,
can also be administered.

The drug will typically be administered in an
appropriate pharmaceutically acceptable carrier
15 having an acoustic impedance similar to water, such
as an aqueous gel. Alternatively, a transdermal
patch such as the one described in the examples can
be used as a carrier. Drug can be administered in
a gel, ointment, lotion, suspension or patch, which
20 can incorporate anyone of the foregoing.

Drug can also be encapsulated in a delivery
device such as a liposome or polymeric
nanoparticles, microparticle, microcapsule, or
microspheres (referred to collectively as
25 microparticles unless otherwise stated). A number
of suitable devices are known, including
microparticles made of synthetic polymers such as
polyhydroxy acids such as polylactic acid,
polyglycolic acid and copolymers thereof,
30 polyorthoesters, polyanhydrides, and
polyphosphazenes, and natural polymers such as
collagen, polyamino acids, albumin and other
proteins, alginate and other polysaccharides, and
combinations thereof. The microparticles can have
35 diameters of between 0.001 and 100 microns,
although a diameter of less than 10 microns is
preferred. The microparticles can be coated or

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formed of materials enhancing penetration, such as
lipophilic materials or hydrophilic molecules, for
example, polyalkylene oxide polymers and
conjugates, such as polyethylene glycol. Liposome
5   are also commercially available.

Administration of Drug.

The drug is preferably administered to the
skin at a site selected based on convenience to the
patient as well as maximum drug penetration. For
10   example, the arm, thigh, or stomach represent areas
of relatively thin skin and high surface area,
while the hands and feet are uneven and calloused.
In the preferred embodiment, drug is applied to the
site and ultrasound and electrical current applied
15   immediately thereafter. Other enhancers can be
applied before, during or immediately after the
ultrasound. Chemical enhancers are preferable
administered during or before ultrasound.

Based on these calculations and experimental
20   data, one can calculate the required dosage and
application regime for treatment of a patient, as
follows. A typical diabetic patient (70 Kg weight)
takes about 12 Units of insulin three times a day
(total dose of about 36 Units per day: cited in
25   'World Book of Diabetes in Practice' Krall, L.P.
(Ed), Elsevier, 1988). If each insulin dose was to
be delivered by sonophoresis in 1 hour, the
required transdermal flux would be 12 U/hour. Note
that 1 unit (1 U) of insulin corresponds
30   approximately to 40 μg of insulin. The transdermal
patch area used in these calculations is 40 cm^2 (the
area of a transdermal FENTANYL™ patch [ALZA
Corporation]). The donor concentrations used in
these calculations are 100 U/ml in the case of
35   insulin (commercially available insulin solution
[Humulin]), 3 x 10^7 in the case of γ-interferon
(typical concentration of interferon solution

recommended by Genzyme Corporation), and 3×10^5 U/ml in the case of erythropoietin [Davis, et al., *Biochemistry*, 2633-2638, 1987].

5 A typical γ-interferon dose given each time to patients suffering from cancer or viral infections is about 5×10^6 U [(i) Grups, et al., *Br. J. Med.*, 1989, 64 (3): 218-220, (ii) Parkin, et al., *Br. Med. J.*, 1987, 294: 1185-1186]. Similar doses of α-interferon and β-interferon have also been shown to 10 enhance the immune response of patients suffering from viral infections and cancer (cited in 'Clinical Applications of interferons and their inducers', Ed. Stringfellow D., Marcel Dekker, New York, 1986). If this interferon dose was to be 15 given by sonophoresis in 1 hour, the required transdermal flux would be 5×10^6 U/hour. Note that 1 unit of γ-interferon corresponds approximately to 1 pg of γ-interferon.

A typical daily erythropoietin dose given 20 subcutaneously to anemic patients is about 400 U (cited in 'Subcutaneous Erythropoietin, Bommer J., Ritz E., Weinreich T., Bommer G., Ziegler T., Lancet, 406, 1988). If this dose was to be delivered in three steps, each involving 25 sonophoresis for 1 hour, the transdermal flux required would be about 140 U/hour. Note that 1 unit of erythropoietin corresponds approximately to 7.6 nanograms of erythropoietin.
Optimal selection of ultrasound parameters, such as 30 frequency, pulse length, intensity, as well as of non-ultrasonic parameters, such as ultrasound coupling medium, can be conducted to ensure a safe and efficacious application using the guidelines disclosed herein as applied by one of ordinary 35 skill in the art.

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Analytes to be Measured.

A variety of analytes are routinely measured in the blood, lymph or other body fluids. Measurements usually require making a puncture in order to withdraw sample. Examples of typical analytes that can be measured include blood sugar (glucose), cholesterol, bilirubin, creatine, various metabolic enzymes, hemoglobin, heparin, vitamin K or other clotting factors, uric acid, carcinoembryonic antigen or other tumor antigens, and various reproductive hormones such as those associated with ovulation or pregnancy. Transdermal drug delivery, in combination with the non-invasive blood analyte measurements, may be used to formulate self-regulated drug delivery methods which provide a close control of the blood concentrations, minimal pain, and better patient compliance. Non-invasive blood analysis method includes extraction of various analytes from the skin's interstitial fluids (where the analytes are present at a concentration proportional to the blood concentration) across the skin into a patch, solution or gel, where their concentration can be measured using biosensors. This method of blood analyte measurements should be particularly useful in the case of diabetic patients who require multiple daily blood glucose measurements.

Measurement of Analytes.

The ultrasound is applied to the skin at the site where the sample is to be collected. A reservoir or collecting container is applied to the site for collection of the sample, which is then measured using standard techniques. The ultrasound conditions are optimized as in the case for drug delivery, to maximize analyte recovery, while maintaining the relative levels of the analyte to other components of the sample. Chemical and/or

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physical enhancers are applied to the site before,
during and after the ultrasound, preferably during
or before the ultrasound.

5   The present invention will be further
understood by reference to the following non-
limiting examples.

EXAMPLE 1:   *In vitro* administration of insulin.

      Materials and methods: *In vivo* as well as *in
vitro* experiments were performed to study the
10   effect of low-frequency ultrasound on the transport
of insulin across the skin. *In vitro* experiments
were performed using human cadaver skin. The skin
was heat stripped by keeping it in water at 60∘C
for two minutes followed by the removal of the
15   epidermis. It was then stored at 4°C in a
humidified chamber. A piece of epidermis was taken
out from the chamber prior to the experiments and
was mounted on the Franz diffusion cell (Crown
Bioscientific Co.) which consists of two
20   compartments, the donor and the receiver
compartment. The human cadaver epidermis
(separated from the dermis by heat-treatment) is
mounted between the two compartments and is
supported by a Nylon mesh (Tetko Inc.) to avoid any
25   damage. The skin was supported by a nylon mesh
(Tetko Inc.) in order to a mimic the fact the skin
in vivo is supported by mechanically strong dermis.
The compartments were then clamped together. The
receiver compartment was filled with 2% BSA (Sigma
30   Chemicals) solution in PBS (Sigma Chemicals) and
the donor solution was filled with 100 U/ml
solution of human recombinant insulin (Humulin
Regular). The ultrasound intensity, *I*, (Spatial
Average Temporal Peak) was calculated from the
35   values of the acoustic pressure amplitude, *P*,
measured using a hydrophone (Bruel and Kjaer) using
the equation, $I = P^2/ 2\rho c$, where ρ is the water

density (1 gm/ml), and c is the velocity of
ultrasound in water [1500 m/s].

Ultrasound was turned ON at a frequency of 20
KHz, an intensity varying in the range of 0 to 1
5   W/cm^2 and 10% duty cycle. Samples (200 μl) were
taken from the receiver compartment every hour to
measure the concentration of insulin in the
receiver compartment. The samples were immediately
frozen and were stored at -20° C till they were
10 analyzed by RIA (Linco Research Co.). Ultrasound
was typically applied for 4 hours and was then
turned OFF. Transdermal insulin flux was followed
for next two hours.

Results: The results are shown in Figures 1A
15 and 1B and demonstrate that substantially greater
transfer of protein through the skin occurs in the
presence of ultrasound. Figures 1a and 1b show the
variation of transdermal insulin flux across the
human skin in vitro. Ultrasound (20 KHz, 125
20 mW/cm^2, 10%) was turned ON at time zero. The
insulin flux increased from below the detection
limit to a value of about 100 mU/cm^2/hr in about 1
hour and stayed almost constant around that value
as long as ultrasound was ON. Upon turning
25 ultrasound OFF, the insulin flux decreases and
achieves a value below our detection limit within 2
hours after turning ultrasound OFF. The skin
permeabilities to insulin at various ultrasound
intensities were calculated from the amount of
30 insulin transported during the first hour of
ultrasound exposure and are shown in Figure 1b.
The sonophoretic permeability varies nearly
exponentially with ultrasound intensity, probably
due to a highly non-linear dependence of cavitation
35 on ultrasound intensity (Apfel, R. E., IEEE Trans.
Ultrason. Ferroelectrics Freq. Control 1986,
UFFC-33, 139).

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Application of ultrasound under these
conditions did not appear to cause any permanent
loss of the barrier properties of the skin. The
transdermal insulin flux (proportional to the slope
5   of the curves shown in Figure 1a) three hours after
turning ultrasound OFF was statistically
insignificant. To further assess the recovery of
the skin barrier properties after sonophoresis,
water transport was measured through the skin
10   *during* and *after* ultrasound exposure (20 KHz, 125
mW/cm^2, 100 msec pulses applied every second).
Transdermal water transport was measured using the
same set-up utilized in the insulin experiments,
except that the donor compartment was filled with a
15   1 μCi/ml solution of radiolabelled water (^{3}H). The
concentration of water in the receiver compartment
was measured using, a scintillation counter.
During sonophoresis, a water permeability
enhancement of 100-fold was observed, of which
20   about 94 ($\pm$ 3)% was recovered within 2 hours after
turning ultrasound OFF and 98 ($\pm$ 1)% was recovered
within 15 hours. These results suggest that
application of ultrasound does not induce any long-
lasting loss of the skin barrier properties.
25   With a transdermal insulin flux of 100
mU/cm^2/hr, it should be possible to deliver
therapeutic doses of insulin transdermally.
Specifically, an insulin dose of about 13 U/h (a
dose comparable to the estimated dose required by a
30   diabetic patient if insulin is administered at a
controlled rate) could be delivered from a patch
having an area of 100 cm^2. Accordingly, ultrasound
intensity should be useful to control transdermal
insulin delivery.
35   **EXAMPLE 2:**   **In vitro transfer of other proteins.**
Methods and materials: The passive skin
permeability to high-molecular weight proteins,

including those mentioned above, *is essentially
zero* (below the detection limit). To assess
whether application of ultrasound enhances
transdermal protein flux, the skin permeability to

5   these proteins in the presence of ultrasound *in
vitro* across human cadaver epidermis in a Franz
Diffusion Cell (Crown Glass Company) was measured.
In separate experiments, the donor compartment of
the diffusion cell was filled with a solution of

10   insulin (100 U/ml, Humulin Regular, Eli Lilly), γ-
interferon (2500 U/ml, Genzyme Corp.), or
erythropoeitin (400 U/ml, Amgen Corp.). Ultrasound
(20 KHz, 100 msec pulses applied every second) was
applied at intensities in the range of 12.5 mW/cm^2-

15   225 mW/cm^2 for 4 hours using an ultrasound
transducer (VCX 400, Sonics and Materials) which
was immersed in the donor solution. The transducer
having an area of about 1 cm^2 was oriented
perpendicular to the skin and placed at a distance

20   of 1 cm from the skin. The concentration of
proteins in the receiver compartment was measured
every hour either by RIA or ELISA. The insulin
concentration in the receiver compartment was
measured every hour by Radioimmuno Assay (performed

25   at Linco Research Inc., St. Charles). The γ--
interferon concentration was measured using ELISA
methods developed by Endogen Inc, and the
erythropoeitin concentration was measured by ELISA
(performed at ARUP, Salt Lake City). Skin

30   permeabilities to proteins were calculated using
the transdermal fluxes measured during the first
hour. The transdermal flux can be calculated using
the equation, $J = DM/\Delta t$, where ΔM is the amount of
protein transported per unit skin area during time

35   Δt. The skin permeabilities, P, can be calculated
from the transdermal flux, J, during the first hour
of ultrasound application using the equation, P

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$=J/\Delta C$, where ΔC is the concentration difference across the skin.

    <u>Results</u>: Ultrasound application induces significant transdermal permeation of insulin, γ-

5   interferon, and erythropoeitin. As demonstrated in Example 1, the human skin permeability at an ultrasound intensity of 225 mW/cm^2 is 3.3×10^{-3} ($\pm$ 35%) cm/h to insulin. The permeability to γ-interferon under similar ultrasound conditions is 8

10    $\times 10^{-4}$ ($\pm$ 22%) cm/h, and that to erythropoeitin is 9.8×10^{-6} ($\pm$ 40%) cm/h. With these skin permeabilities, it should be possible to deliver these proteins transdermally at a therapeutically relevant rate. The ability of sonophoresis to

15   deliver other macromolecules may be estimated based on their sonophoretic skin permeability which needs to be measured experimentally (generally decreases with increasing molecular size) and the required therapeutic dose of these macromolecules.

20   **EXAMPLE 3:**   **Transdermal glucose extraction by sonophoresis in vitro.**

    Application of low-frequency ultrasound can be used to extract glucose across the skin, thus making non-invasive transdermal blood glucose

25   monitoring potentially feasible.

<u>Materials and Methods</u>:

    In Vitro Transdermal Transport Measurements:

    Transdermal transport of a ^{14}C labeled (New England Nuclear) as well as non-labeled (Sigma

30   Chemicals) was studied in the presence as well as in the absence of low-frequency ultrasound. The permeability experiments were performed *in vitro* using human cadaver skin obtained from local hospitals. The skin was heat stripped by keeping

35   the full-thickness skin in water at 60°C for two minutes followed by the removal of the epidermis. The skin was then stored at 4°C in a humidified chamber for up to 2 weeks. A piece of the

epidermis was taken out from the chamber prior to
the experiments and was mounted on a Franz
diffusion cell (Crown Glass Co., FDC 200). The
Franz diffusion cell consists of two compartments,

5   the donor and the receiver compartments, with the
stratum corneum facing the donor compartment. The
skin was supported by a nylon mesh (Tetko, Inc.) to
avoid any damage due to possible mechanical
oscillations upon ultrasound application. The

10   donor and receiver compartments were then clamped.
The receiver compartment was filled Phosphate
Buffer Saline (PBS, phosphate concentration = 0.01
M, NaCl concentration = 0.137M) (Sigma Chemicals
Co.) The donor compartment was filled with a

15   solution of either radiolabelled glucose (1 mCi/ml)
or non-labeled glucose (concentration in the range
of 50 mg/dL to 300 mg/dL) in separate experiments.
The concentration of the permeant in the receiver
compartment was measured every 5 minutes using a

20   scintillation counter (model 2000 CA, Packard) in
the case of radiolabelled glucose and using a
commercially available kit (Sigma Chemicals) in the
case of unlabeled glucose.

       Ultrasound was applied using a sonicator (VCX

25   400, Sonics and Materials) operating at a frequency
of 20 KHz. The ultrasound intensity was measured
using a hydrophone (Model 8106, Bruel and Kjaer).
RESULTS

       Figure 2 shows the glucose concentration in

30   the donor compartment (represented as percent of
the glucose concentration in the receiver
compartment) attained at different times during
transdermal glucose extraction experiment. The
figure shows that even a 5 minute ultrasound

35   application (20 KHz, 125 mW/cm^2, continuous) results
in a significant glucose transport across human
skin *in vitro*. Specifically, the glucose

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concentration in the donor compartment after 5
minutes of sonophoresis is about 0.5% of that in
the receiver compartment. After 10 minutes, the
glucose concentration in the donor compartment was

5   about 2% of that in the receiver compartment. The
glucose concentration in this range can be measured
in situ using glucose sensing electrodes, and can
be calibrated to indicate actual blood glucose
levels.

10   The amount of glucose extracted by
sonophoresis under a given condition varies in the
case of skin obtained from different donors
(typical variation 40%(SD). However, the variation
in the case of skin obtained from the same donor is

15   only about 13%, thus indicating that it should be
possible to achieve reliable estimates of glucose
concentrations based on transdermal glucose
extraction after performing calibration in vivo on
the patient's skin.

20   Additional experiments were performed to
assess whether the amount of glucose transported by
sonophoresis is proportional to the glucose
concentration in the receiver compartment. In
separate experiments, glucose concentration in the

25   receiver solution was varied from 50 mg/dL to 350
mg/dL (typical variation in the blood glucose level
of a diabetic patient) and performed sonophoresis
using ultrasound (20 KHz, 125 mW/cm^2, continuous)
for 10 minutes. Figure 3 shows that the glucose

30   concentration attained in the donor compartment 10
minutes after sonophoresis (represented as
percentage of the glucose concentration in the
receiver compartment) increased from 0.5 mg/dL to
6.5 mg/dL as glucose concentration in the receiver

35   compartment increased from 50 mg/dL to 350 mg/dL.
The line shown in Figure 3 represents the best fit.
These results show that the amount of glucose

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extracted across human skin is proportional to the
glucose concentration under the skin, thus
indicating that transdermal glucose extraction by
sonophoresis could be potentially used for blood
5   glucose measurement.

    EXAMPLE 4:   **Effect of Ultrasound Intensity and
Chemical Enhancers on Transdermal
Transport.**

    Materials

10   Human cadaver skin from the chest, back, and
abdominal regions was obtained from local hospitals
and the National Disease Research Institute.   The
skin was stored at -80°C until usage.   The epidermis
was separated from the full-thickness tissue after
15   immersion in 60°C water for 2 minutes.   Heat-
stripped skin was stored at 5°C and 95% humidity for
up to 1 week prior to usage. ^{3}H-corticosterone, ^{3}H-
dexamethasone, ^{3}H-testosterone, and ^{14}C-linoleic
acid were obtained from DuPont, New England
20   Nuclear.   Non-radiolabeled corticosterone (95%),
dexamethasone (99+%), testosterone (99+%), and
linoleic acid (99%) were obtained from Sigma
Chemical.   Glycerol trioleate (99+%) and
Polyethylene glycol 200 Dilaurate (99+%) were
25   obtained from Henkel.   Isopropyl myristate (98%)
was obtained from Aldrich Chemicals and butanediol
(98%) was obtained from ISP Technologies.   Ethanol
was obtained from Pharmco Products.

    A.   **Methods for Passive permeability**
30   **experiments**

    The passive permeabilities (e.g., permeability
without the application of ultrasound) of
corticosterone, dexamethasone, and testosterone
through human skin were measured using trace
35   quantities of radiolabelled drug.   The
radiolabelled drugs were rotary evaporated in order
to remove the ethanol in which they were shipped
and any tritium which had reverse exchanged onto

35

it. The radiolabelled drugs were then redissolved
in an enhancer formulation to a typical
concentration of 1 μCi/ml, and added to the donor
chamber of the permeation cell. Passive permeation

5   experiments were performed using stirred side-by-
side diffusion cells (Crown Glass, #DC-100B). The
receiver compartment always contained pH 7.4
phosphate buffer saline (PBS, phosphate
concentration = 0.01 M, NaCl concentration = 0.137

10   M) (Sigma Chemical Co.). The concentrations of
radiolabelled drug in the donor and receiver
compartments were measured using a scintillation
counter (model 2000 CA, Packard Instruments). A
minimum of three experiments were performed with

15   each enhancer formulation.
        The permeability values were calculated under
steady-state conditions from the relationship $P = (dN_r/dt)/(AC_d)$ where A is the surface area of the
skin sample, C_d is the drug concentration in the

20   donor chamber, and N_r is the cumulative amount of
drug which has permeated into the receptor chamber.
The experimentally observed lag-times for the
permeation experiments were 1 to 6 hours for
corticosterone, 2 to 8 hours for dexamethasone, and

25   less than 1 hour for testosterone. The variability
of the individual permeability values were
consistent with previously established inter-
subject variability of the human skin permeability
of 40%, as reported by Williams, et al., *Int. J.*

30   *Pharm.* 86, 69-77 (1992). The passive permeability
enhancements, E_p, were calculated relative to the
passive permeability from PBS according to Eq. (1):

$$\epsilon_p \equiv \frac{P(enhancer)}{P(PBS)} \qquad (1)$$

35

36

where $P(enhancer)$ is the drug permeability from a
given enhancer, and $P(PBS)$ is the drug permeability
from PBS.   The fluxes from saturated solutions,
J^{sat}, were calculated from $J^{sat}=PC^{sat}$, where C^{sat} is the
5   drug solubility in the formulation. Flux
enhancements, E_j, were calculated using Eq. (2),

$$\epsilon_J \equiv \frac{J(enhancer)}{J(PBS)} \qquad (2)$$

10   where $J^{sat}(enhancer)$ and $J^{sat}(PBS)$ are the drug fluxes
from saturated solutions of enhancer and PBS,
respectively.

The results of the passive corticosterone
transport experiments are shown in Table 1.

TABLE 1: Corticosterone Transport Properties With Chemical Enhancers

Enhancer	Steady-State Permeability, P (cm/hr x10⁵)	Permeability Enhancement, ε_p	Solubility, C^{sat} (mg/ml)	Saturated Flux, J^{sat} (mg/cm²/hr x10⁵)	Flux Enhancement, ε_J
Phosphate buffer	10±32%	1.0	0.12	1.2	1.0
PEG 200 Dilaurate	2.4±29%	0.24	0.94	2.2	1.9
Isopropyl Myristate	7.0±38%	0.70	0.77	5.4	4.5
Glycerol trioleate	7.1±29%	0.71	0.14	1.0	0.8
50% Ethanol 50% Buffer	5.2±21%	0.52	9.2	48	40
Linoleic acid in 1:1 Ethanol:Buffer	87±34%	8.7	12.4	1080	903

38

The results reveal that the enhancer
formulations ·fall into two groups. The first group
of chemical enhancers, PEG, IM, and GT, produced
only modest effects upon corticosterone transport,

5   while the second group, 50% Ethanol and LA/Ethanol,
had a significant impact. With respect to the
first group, PEG and IM are better solubilizers of
corticosterone, with measured solubilities of 0.94
and 0.77 mg/ml. The solubility of corticosterone

10   in PBS is considerably lower, 0.12 mg/ml, but is
similar to the solubility of corticosterone in GT,
0.14 mg/ml, as shown in Table 1. These increases
in solubility for PEG and IM, however, do not
translate into significantly greater saturated

15   fluxes than that from PBS. This is due to the
decreases in the corticosterone permeabilities from
PEG and IM relative to that from PBS.
Specifically, the measured corticosterone
permeability from PBS is 1.0×10^{-4} cm/hr, while

20   those from PEG and IM are only 2.4×10^{-5} and 7.0×10^{-5} cm/hr, as shown in Table 1. As a result, the
flux enhancements from PEG and IM are moderate, 1.9
and 4.5, respectively. GT, whose corticosterone
solubility is similar to that of PBS, also has a

25   corticosterone permeability, 7.1×10^{-5} cm/hr, which
is similar to that of PBS, 1.0×10^{-4} cm/hr, as
shown in Table 1. Thus, the corticosterone flux
from a saturated solution of GT, 1.0×10^{-5}
mg/cm²/hr, is similar to that from saturated PBS,

30   1.2×10^{-5} mg/cm²/hr. In summary, the differences
in the solubilities, permeabilities, and fluxes of
corticosterone from PBS, PEG, IM, and GT are all
relatively moderate.

In contrast, 50% Ethanol and LA/Ethanol
35   significantly increase the transdermal transport of
corticosterone. The permeability of corticosterone
from 50% Ethanol, 5.2×10^{-5} cm/hr, is nearly two-

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fold lower than that from PBS, 1.0×10^{-4} cm/hr, and
in the same range as those from PEG, IM, and GT as
well. However, 50% Ethanol is a very effective
solubilizer. 9.2 mg/ml is the corticosterone

5 solubility in 50% Ethanol, which is nearly 100-fold
greater than that in PBS, 0.12 mg/ml, as shown in
Table 1. This greater degree of solubilization
results in a significantly greater flux of 4.8×10^{-4} mg/cm^2/hr, which is a factor of 40 greater than of

10 that from PBS.

Even more effective is LA/Ethanol, which is
50% ethanol (v/v) saturated with linoleic acid.
Table 1 shows that the corticosterone permeability
from LA/Ethanol is 8.7×10^{-4} cm/hr. Note that all

15 of the other formulations have lower permeabilities
than from PBS, while the permeability from
LA/Ethanol is nine-fold greater. The permeability
enhancement achieved through the mere addition of
linoleic acid to 50% Ethanol is 17-fold, clearly

20 showing the effectiveness of the unsaturated fatty
acid in increasing transport. Addition of linoleic
acid to 50% Ethanol increases the corticosterone
solubility to 12.4 mg/ml in LA/Ethanol from 9.2
mg/ml in 50% Ethanol alone, as shown in Table 1.

25 Addition of the oily linoleic acid tends to make
the solution more hydrophobic and slightly less
polar, which is a more attractive environment for
corticosterone. The combination of permeation
enhancement and increased corticosterone solubility

30 arising from the use of linoleic acid in 50%
Ethanol combine to yield saturated drug fluxes of
1.1×10^{-3} mg/cm^2/hr, which is 903-fold greater than
from water and more than 20-fold greater than from
50% Ethanol (i.e., without the linoleic acid).

35 In order to examine the impact of linoleic
acid on corticosterone transport without coupling
it with 50% Ethanol, control experiments were

40

performed in which corticosterone permeabilities
were measured from PBS saturated with linoleic
acid. The resulting corticosterone permeability,
1.1 x10^{-4} ($\pm$ 0.34 x 10^{-4}) cm/hr, is indistinguishable

5   from the PBS permeability of 1.0 x 10^{-4} ($\pm$ 0.32 x
10^{-4}) cm/hr reported in Table 1. Clearly, ethanol
and linoleic acid are each ineffective in
increasing corticosterone permeability when
utilized individually, but when utilized together,

10   they yield a substantial degree of enhancement.

   B.   **Effect of Ultrasound in Combination with**
        Chemical Enhancers

   Ultrasound was applied under therapeutically
approved conditions (1.4 W/cm^2, 1 MHz, continuous)

15   for 24 hours using a sonicator (Sonopuls 463,
Henley International). The ultrasound transducer
was located approximately 3 cm from the surface of
the skin. Permeation experiments were performed
using customized side-by-side diffusion cells

20   having a skin area of 3.1 cm^2 and a receiver
compartment volume of 7.5 ml. Samples were taken
from the receiver compartment over 24 hours. The
concentrations of radiolabelled drug in these
samples, as well as in the donor compartment, were

25   measured using a scintillation counter (model 2000
CA, Packard Instruments). Three or more
experiments were performed using each of the
chemical enhancers listed above. PBS was always
used in the receiver compartment. Sonophoretic

30   permeabilities were constant once steady-state was
achieved. The drug permeabilities in the presence
of ultrasound were $P_{us} = (dN_r/dt)/(AC_d)$. The
exception to this observation was the combination
of therapeutic ultrasound and SA/Ethanol, with

35   which the corticosterone permeability continually
increased.

41

Studies with therapeutic ultrasound 1 MHz
performed at an intensity of 2.0 W/cm^2 by
Mitragortri, et al. *J. Pharm. Sci.* 84, 697-706
(1995) showed that the continuous application of
5   ultrasound increased transdermal permeabilities,
but only for a short period of time. After 5 to 6
hours, the sonophoretic enhancement abated and the
observed permeabilities returned to the passive
values. This sonophoretic enhancement was found to
10 be caused by cavitation within the skin, where
cavitation is defined as the growth and oscillation
of air bubbles which disorder the stratum corneum
lipid bilayers. In the present study, ultrasound
was applied at a lower intensity, 1.4 W/cm^2, and 1
15 MHz. Sonophoretic permeability enhancements lasted
for extended periods of time for corticosterone,
dexamethasone, and testosterone at this intensity.
The elevated transdermal permeabilities resulting
from the continuous application of ultrasound at
20 1.4 W/cm^2 were maintained for up to 48 hours, the
longest sonophoretic experiment performed. As a
control, the permeability of corticosterone was
measured with therapeutic ultrasound applied at 2.0
W/cm^2. As was previously found and reported for
25 estradiol, the permeation enhancement lasted for
only 5 to 6 hours. This difference in the duration
of the sonophoretic enhancements resulting from
differences in the ultrasound intensity is probably
due to the change in the magnitude of the
30 cavitation activity. Since cavitation results in
the degassing of the system, the greater ultrasound
intensity results in an accelerated degassing of
the system, which in turn results in shorter
duration of the permeability enhancements, as was
35 observed.

The transmission efficiency of ultrasound
through the various enhancers was measured using a

hydrophone (model PZT 54, Specialty Engineering Associates) coupled to a hydrophone preamplifier (model A17DB, Specialty Engineering Associates), and connected to an oscilloscope (model 7623 A,

5   Hewlett Packard). The hydrophone was calibrated by Sonic Technologies. The ultrasound intensity in the diffusion cell was first measured with both probes submerged in the formulation and the hydrophone in close proximity to the ultrasound

10   transducer. The ultrasound intensity was subsequently measured with the transducer in the donor chamber of the permeation cell and the hydrophone in the receiver chamber 5-6 cm away from the transducer. No differences in the measured

15   intensities were observed for any formulation, indicating that all of the chemical enhancer formulations examined were uniformly efficient in transmitting ultrasound.

      The uptake of ^{14}C-linoleic acid into human SC

20   was measured with and without the application of therapeutic ultrasound (1.4 W/cm^2, 1 MHz, continuous). SC was separated from heat stripped epidermis by soaking the epidermis in 0.5% Trypsin solution overnight at 5°C. The SC was cleaned with

25   water, rinsed in cold hexane to remove any exogenous lipids, and lyophilized for at least 24 hours to remove all water. Dried pieces of SC were sectioned into pieces approximately 10 mg in weight and weighed. These SC pieces were place in a glass

30   chamber mounted on an ultrasound probe containing 3 ml of solution of ^{14}C-linoleic acid in LA/Ethanol and sealed. 25 μl samples were taken from the chamber periodically, and counted with the liquid scintillation counter.

35   Ultrasound is effective in increasing the permeability of corticosterone from all of the formulations examined, as shown in Table 2.

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TABLE 2:   Ultrasound-Mediated Permeability Enhancement of Corticosterone

Enhancer	Permeability Without Ultrasound, P (cm/hr x10^5)	Permeability With Ultrasound, P_{us} (cm/hr x 10^5)	Ultrasound Enhancement $\varepsilon_{p,us}$
Phosphate buffer	10±32%	50±39%	5.0
PEG 200 Dilaurate	2.4±29%	4.5±24%	1.9
Isopropyl Myristate	7.0±38%	25±34%	3.6
Glycerol trioleate	7.1±29%	9.3±36%	1.3
50% Ethanol 50% Buffer	5.2±21%	9.8±22%	1.9
Linoleic acid in 50:50 Ethanol:Buffer	87±34%	≥1260±50%	≥14.4

*Experiments were performed on skin samples which had been utilized for passive permeability measurements, such that the lag-times were already surpassed.

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The values of the sonophoretic permeability
enhancements, $E_{p,us}$, defined as

$$\epsilon_{p,us} \equiv \frac{P_{us}}{P} \qquad (3)$$

are all greater than unity. Note that $E_{p,us}$, is the
ratio of the ultrasound mediated permeability in a
given formulation to the passive permeability in
the *same* formulation, and hence is a measure of the
10   effectiveness of ultrasound with that particular
formulation. Table 2 shows that ultrasound
mediated permeabilities for the first group of
enhancers, PBS, PEG, IM, and GT, are all moderate,
ranging from 1.3 for GT to 5.0 for water. The
15   sonophoretic enhancement from 50% Ethanol, 1.9, is
also moderate in its value. The most significant
sonophoretic enhancement is obtained with the
formulation containing linoleic acid, LA/Ethanol.
The sonophoretic permeability from LA/Ethanol is
20   1.3×10^{-2} cm/hr , which is a factor of 14 greater
than the passive corticosterone permeability from
LA/Ethanol. These results clearly show that
ultrasound is effective in increasing transdermal
drug permeation when utilized with both aqueous as
25   well as non-aqueous formulations.

Sonophoretic Saturated Fluxes and Enhancement

The values of the ultrasound mediated
corticosterone fluxes from saturated solution, J_{us},
where $J_{us} = P_{us}C^{sat}$, are listed in Table 3.

TABLE 3: Enhancement of Corticosterone Transport by Chemical Enhancers and Ultrasound

Enhancer	Sonophoretic Saturated Flux, J_{us} (mg/cm²/hr x10⁵)	Sonophoretic Saturated Flux Enhancement, $\varepsilon_{J,us}$
Phosphate buffer	6.0	5.0
PEG 200 Dilaurate	4.2	3.5
Isopropyl Myristate	20	16
Glycerol trioleate	1.3	1.1
50% Ethanol 50% Buffer	90	75
1 % Linoleic acid in 50:50 Ethanol:Buffer	≥15,600	≥13,000

The fluxes from PBS, PEG, IM and GT are all
fairly low, ranging from 1.3×10^{-5} mg/cm²/hr for GT
to 6.0×10^{-5} mg/cm²/hr for PBS. The flux from PBS
is greater than those from PEG, IM, and GT, due to
greater sonophoretic permeability enhancement for
PBS, shown in Table 2. While the flux from 50%
Ethanol is 15-fold greater than that from PBS, 9.0×10^{-4} mg/cm²/hr, it is still a relatively low
value. Table 3 shows that the use of LA/Ethanol
and therapeutic ultrasound yields a flux greater
than or equal to 0.16 mg/cm²/hr, which is more than
two orders of magnitude greater than that from 50%
ethanol with ultrasound. Also listed in Table 3
are the sonophoretic saturated flux enhancements,
$E_{J,us}$, which is defined as

$$(4) \quad \epsilon_{J,us} \equiv \frac{P_{us} \,(\text{enhancer})\, C^{sat}\,(\text{enhancer})}{P \,(\text{PBS})\, C^{sat}\,(\text{PBS})}$$

$E_{J,us}$ represents the flux enhancement relative
to the passive flux from PBS, used to establish the
base line. Moderate flux enhancements are observed
for PBS, PEG, GT, and 50% Ethanol, ranging from 1.1
for GT to 75 for Ethanol. LA/Ethanol again is seen
to provide tremendous flux enhancement, 13,000-fold
more so than from passive PBS. This enormous
enhancement is the result of the combination of
ethanol, linoleic acid, and therapeutic ultrasound.
Ethanol and water (1:1, v/v) greatly increases the
saturated concentration of corticosterone (Table
1). Linoleic acid increases both the
corticosterone solubility in 50% Ethanol as well as
the corticosterone permeability, while ultrasound
further increases the drug permeability when
applied in conjunction with linoleic acid.

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C.   Solubility measurements in chemical
      enhancers

      In separate studies, excess unlabeled
corticosterone, dexamethasone, and testosterone
5   were each placed in several milliliters of enhancer
and thoroughly mixed. After equilibration for a
minimum of 24 hours the solutions were removed,
centrifuged at 1000 rpm (212 x g) for 10 minutes,
and sampled. Samples were diluted to an
10   appropriate concentration for high performance
liquid chromatographic (HPLC) analysis utilizing
the appropriate HPLC mobile phase. Methanol and
water (60:40 v/v) was utilized as the mobile phase
for corticosterone and testosterone, and
15   acetonitrile and water (35:65 v/v) was utilized for
dexamethasone. The mobile phases were filtered
with 0.22 μm PTFE hydrophobic filters and degassed
prior to usage. A μ-Bondapak C18 (30 cm x 4 mm,
i.d.) HPLC column was used. The sample volume was
20   40 μl and the mobile phase flow rates were 1.4
ml/minute (corticosterone) and 2.0 ml/min.
(corticosterone, dexamethasone, and testosterone).
An ultraviolet detector (Waters 490) was used at a
wavelength of 240 nm for all three drugs.
25   Standards were prepared by diluting a stock
solution of unlabeled drug, prepared by weight,
with the mobile phases. Experiments performed in
triplicate had a standard deviation of 1%.

TABLE 4: Passive and Ultrasound-Mediated Transport of Dexamethasone and Testosterone

Drug	Enhancer	Passive Permeability P (cm/hr x10^5)	Solubility C^{sat} (mg/ml)	Saturated Flux, J^{sat} (mg/cm²/hr x 10^5)	Sonophoretic Permeability P$_{us}$ (cm/hr x10^5)	Sonophoretic Saturated Flux, J$_{us}$ (mg/cm²/hr x 10^5)
Dexamethasone	Phosphate buffer	6.4±40%	0.10	0.66		
	50% Ethanol 50% Buffer	1.7±23%	2.39	4.0		
	Linoleic Acid in 50% Ethanol	217±42%	4.36	945	600±8%	2610
Testosterone	Phosphate buffer	536±17%	0.023	12.3		
	50% Ethanol 50% Buffer	5.5±8%	6.37	35.2		
	Linoleic Acid in 50% Ethanol	64±24%	8.2	525	449±52%	3680

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In order to probe the generality of the
effectiveness of LA/Ethanol alone and the
combination of ultrasound and LA/Ethanol in
enhancing corticosterone transport, experiments
5   were performed with two additional model drugs,
dexamethasone and testosterone. Passive
permeability and solubility measurements were made
with PBS, 50% Ethanol, and LA/Ethanol, as described
above. Ultrasound mediated transport was also
10   measured with LA/Ethanol and ultrasound with both
dexamethasone and testosterone, as this was the
most effective enhancement combination observed in
the corticosterone experiments. The results of
these experiments are shown in Table 4.
15   The solubility of dexamethasone in PBS is 0.10
mg/ml, which is similar to the solubility of
corticosterone in PBS, 0.12 mg/ml. This is not
surprising since dexamethesone and corticosterone
have a similar degree of hydrophobicity, as
20   revealed by their similar octanol/water partition
coefficients of 97 for dexamethasone and 87 for
corticosterone (Hansch and Leo, "Substitutent
Constants for Correlation Analysis in Chemistry and
Biology" (1979)). Testosterone is more hydrophobic
25   than corticosterone and dexamethasone, as indicated
by an octanol/water partition coefficient of 2100,
has a lower solubility in PBS, 0.0234 mg/ml.
Dexamethasone and testosterone are much more
soluble in 50% Ethanol than PBS, 2.39 mg/ml and
30   6.37 mg/ml, and even more soluble in LA/ethanol,
4.36 mg/ml and 8.2 mg/ml respectively. The
relative increases in drug solubility for all three
drugs, corticosterone, dexamethasone, and
testosterone, are shown in Figure 4. Solubilities
35   of corticosterone, dexamethasone, and testosterone
in PBS, 50% Ethanol, and LA/ethanol were measured
using HPLC. The solubilities of these drugs in

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PBS, 0.12 mg/ml, 0.10 mg/ml, and 0.023 mg/ml
respectively, are 77, 44, and 360 fold lower than
the solubilities in 50% Ethanol. Corticosterone,
dexamethasone, and testosterone are even more

5   soluble in 50% Ethanol saturated with linoleic acid
(LA/Ethanol) by an average factor of 1.5.

The experimentally measured permeability of
dexamethasone from PBS is 6.4×10^{-5} cm/hr, as shown
in Table 4. This value is relatively low, yet the

10   permeability from 50% Ethanol is even lower at 1.7
$\times 10^{-5}$ cm/hr. The permeability of testosterone from
PBS is 5.4×10^{-3} cm/hr, but decreases by nearly two
orders of magnitude to a value of 5.5×10^{-5} cm/hr
when measured from 50% Ethanol. Similar drops have

15   been observed for corticosterone, as shown in Table
1, as well as for estradiol (Liu, et al.
Pharmaceutical Research 8, 938-944 (1991)). These
permeability decreases are a result of the
decreased partitioning of the drugs into the skin.

20   Since 50% ethanol has a lesser degree of polarity
than does water, it is more attractive environment
relative to PBS, and shifts the equilibrium drug
distributions away from the skin and towards the
donor solution. Since the skin permeability is

25   proportional to the partition coefficient, skin
permeabilities will decrease as the donor solution
becomes less polar than PBS and a better
solubilizer of the drugs. The permeability of
dexamethasone from LA/Ethanol, 2.2×10^{-3} cm/hr, is

30   significantly greater than that from PBS, 6.4×10^{-5}
cm/hr, as was also observed with corticosterone.
However, the permeability of testosterone from
LA/Ethanol, 6.4×10^{-3} and 5.3×10^{-3} mg/cm^2/hr, are
within a factor of two of the corticosterone

35   saturated flux, 1.1×10^{-2} mg/cm^2/hr.

Dexamethasone and testosterone sonophoretic
transport measurements were made with LA/Ethanol,

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which is the most effective combination that was determined through the corticosterone experiments. The results of these experiments are shown in Table 4. As with corticosterone, the sonophoretically

5   enhanced permeabilities of dexamethasone and testosterone increased over time. However, unlike the corticosterone experiments, these observed permeabilities become steady after 12 to 15 hours, enabling true steady-state measurements to be made:

10   6.0×10^{-3} cm/hr and 4.5×10^{-3} cm/hr for dexamethasone and testosterone, respectively. As with corticosterone, tremendous sonophoretic saturated fluxes of 2.6×10^{-2} mg/cm^2/hr and $3.7 \times$ 10^{-2}/hr are obtained for dexamethasone and

15   testosterone.

Figure 5 shows that the permeation enhancement resulting from the use of linoleic acid is dependent upon the drug examined and the size of that drug. Permeability enhancements for

20   testosterone (288.4 Da), corticosterone (346.5 Da), and dexamethasone (392.5 Da) through (1) the addition of linoleic acid to 50% ethanol and (2) the addition of linoleic acid to 50% ethanol with the continuous application of therapeutic

25   ultrasound relative to the permeabilities from 50% ethanol alone were observed. The enhancements from linoleic acid bear a distinct size dependence, with the larger compounds having larger enhancements. Enhancements with linoleic acid and therapeutic

30   ultrasound bears an analogous size dependence with greater enhancements observed for larger compounds. The permeation enhancements resulting from linoleic acid, (the ratio of the permeability from LA/Ethanol and the permeability from 50% ethanol)

35   alone, are 12 for the smallest drug (testosterone, 288.4 Da), 17 for corticosterone (346.5 Da), and 130 for the largest drug (dexamethasone, 392.5 Da).

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The enhancement effects of three different
enhancers, capric acid (J), lauric acid (B), and
Neodecanoic acid (H), upon the human skin
permeabilities of benzoic acid (122 Da),
5   testosterone (288 Da), naloxone (328 Da), and
indomethacin (359 Da), as compared with propylene
glycol are shown in Figure 6a. These enhancements
exhibit a clear size dependence, with the larger
compounds being enhanced to a greater degree than
10 the smaller compounds. The line is drawn to guide
the eye. The permeability values were originally
reported by Aungst et al.
Figure 6a shows the permeability enhancements
plotted as a function of the molecular weight of
15 the drug. Figure 6a also shows that the variations
in enhancement of a given drug from using the
different enhancers extends to be less than the
variation of enhancements between the different
drugs.
20   The enhancement of Azone upon the
permeabilities of ethanol (46 Da), butanol (74 Da),
corticosterone (346 Da), and hydrocortisone (362
Da) from aqueous solutions through hairless mouse
stratum corneum are shown in Figure 6b. The skin
25 was pretreated by spraying 0.8 mg/cm^2 of Azone upon
it. The degree of permeability enhancement
correlates with the size of the solute. The
permeability values were originally reported in
graphical form by Lambert et al.
30   Fluidization of the stratum corneum lipid
bilayers can increase the partition coefficient
between the bilayers and the donor medium in
addition to increasing diffusion. Since
partitioning is a function of the chemical nature
35 of a solute (i.e., the hydrophobic/hydrophilic
nature of a solute) and not an independent function
of molecular weight, the partitioning effect would

only tend to obscure the size dependence of
enhancement. Linoleic acid and the other chemical
enhancers may increase drug transport through
alternate pathways, often referred to as aqueous

5 pores. The passive skin permeabilities of
hydrophilic compounds, which are thought to diffuse
through these aqueous pores, exhibit a much weaker
size dependence than that of hydrophobic compounds.
While this size dependence is moderate, as are

10 those for fluid phase phospholipid bilayers and
bulk oil phases, fatty acids have been shown to
interact predominantly with the intercellular
lipids. Ethanol alone can also enhance transport
of both hydrophobic and hydrophilic compounds

15 through these aqueous pore pathways, although high
concentrations of ethanol are needed (i.e.
approximately 75% v/v). Ghanem et al., *Int. J.
Pharm*. 78, 137-156 (1992) also report that lipoidal
compounds in solutions of 50% ethanol or less

20 permeate the SC primarily through the lipoidal
domain. This indicates that the combination of 50%
ethanol and linoleic acid may make the aqueous pore
pathway more effective. If the linoleic acid
worked with the 50% ethanol solution to facilitate

25 aqueous pore transport, the passive permeabilities
from LA/Ethanol would be expected to be essentially
constant and independent of size. Tables 1 and 4
show that this is not the case.

The results of sonophoretic enhancement

30 experiments conducted over the last four decades
for more than a dozen different drugs, ranging in
size from 138 Da (salicylic acid) up to 453 Da
(fluocinolone acetonide) were collated. These
studies include both *in vitro* and *in vivo*

35 experiments. While some studies quantified the
degree of enhancement, others simply reported
whether or not sonophoretic enhancement was

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observed. No sonophoretic enhancement has been
observed for drugs smaller than 250 Da whereas
sonophoretic enhancement has been observed for
compounds larger than 250 Da, with the lone
5   exception of progesterone. Sonophoretic
enhancement correlates very well with the drug
passive diffusion coefficient, which in turn is a
strong function of molecular weight.

      Figures 7a and 7b graph the variation of the
10   permeability over time of the corticosterone
permeability through human skin from (Figure 7a)
PBS and (Figure 7b) LA/Ethanol in the presence of
therapeutic ultrasound (1 MHz, 1.4 W/cm²). The
corticosterone permeability from PBS is maintained
15   at the steady-state value of the duration of the 24
experiment once the lag-time period is surpassed.
The corticosterone permeability from LA/Ethanol, on
the other hand, continues to increase over time.
The typical error of the data points is 3%. The
20   lines are drawn to guide the eye.

      The sonophoretically enhanced permeabilities
were constant over time with PBS, PEG, IM, GT, and
50% Ethanol, as shown in Figure 7a for a typical
corticosterone experiment from PBS. After an
25   initial lag time of several hours, the permeability
remains constant for the duration of the 24 hour
experiment. However, when ultrasound was applied
in conjunction with LA/Ethanol, the corticosterone
permeability continually increased. Figure 7b
30   shows the results of one such experiment, wherein
the fraction of corticosterone transported across
the skin is plotted versus time. Whereas steady-
state conditions are defined by linear slope on
such a plot, the slope in Figure 7b continually
35   increases. This continual increase in
corticosterone permeability was observed in every
ultrasound mediated experiment performed with

55

corticosterone and the LA/Ethanol formulation
(n=6).

A steady-state permeability can not be
directly measured in the ultrasound mediated
5   permeation experiments with LA/Ethanol due to the
lack of a linear profile in Figure 7b. The
permeability value listed in Table 2 for this
condition is the average of the permeabilities
observed at the 24 hour mark of the experiment.
10   While this value is not a steady-state
permeability, it does constitute a *lower bound* on
the true steady-state permeability. This value,
1.3×10^{-2} cm/hr, is large relative to the other
corticosterone permeabilities, and is 126-fold
15   greater than the passive permeability from PBS
alone. The true steady-state permeability is
greater than or equal to 1.3×10^{-2} cm/hr and the
sonophoretic enhancement is greater than or equal
to 14. The slightly elevated error associated with
20   this value, a standard deviation of 50%, is also a
result of the fact that the permeabilities were
calculated from non-linear portions of the flux
profiles.

The ultrasound mediated experiments performed
25   with 50% ethanol (without linoleic acid) exhibited
a constant permeability after the initial lag time.
The results shown in Figure 7b are dependent upon
the combined application of linoleic acid and
therapeutic ultrasound. This relationship was
30   further probed in a set of experiments with
corticosterone in LA/ethanol in which ultrasound
was applied for the first eight hours of the
experiments, after which the ultrasound was turned-
off. Variation of the permeability over time of
35   the corticosterone permeability through human skin
from LA/Ethanol with the discontinuous application
of therapeutic ultrasound (1 MHz, 1.4 W/cm^2) was

measured. Ultrasound was applied for the first
eight hours of the experiment, at which time it was
turned off. In contrast with the results of Figure
7b in which ultrasound was applied continuously and
the permeability continued to rise for the entire
24 hour period, the corticosterone permeability
increases up to the point at which the ultrasound
is discontinued, at which point it remains constant
for the remainder of the experiment. The typical
error of the data points is 3%.

EXAMPLE 5:   **Transdermal drug delivery and**
extraction of gluose using
ultrasound in combination with
additional force fields.

Materials and Methods:

In vitro experiments were performed to study
the effect of ultrasound in combination with
electric currents on the delivery of calcein and
extraction of glucose across human cadaver skin.
The skin was heat stripped by keeping it in water
at 60°C for two minutes, followed by the removal of
the epidermis. The skin was then stored at 4°C in a
humidified chamber. A piece of epidermis was taken
out from the chamber prior to the experiments, and
was mounted on a Franz diffusion cell (Crown
Bioscientific Co.) which consists of a donor and a
receiver compartment. The skin was supported by a
nylon mesh (Tetko Inc.) to minimize mechanical
oscillations during ultrasound application. The
donor and receiver compartments were then clamped
together. Two Ag/AgCl electrodes were introduced
in the donor and the receiver compartment for the
application of electric fields. The receiver
compartment was filled with a solution of
radiolabeled glucose in phosphate buffer saline
(Sigma Chemicals), and the donor solution was
filled with a calcein solution (Sigma Chemicals).

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The ultrasound transducer was located at a
distance of about 1 cm from the skin. The
ultrasound was turned ON at a frequency of 20 KHz
and intensity of 125 mW/cm^2 for 10 minutes.

5   Electric current (0.1 mA/cm^2) was applied across the
skin in a few experiments for 70 minutes with anode
(electrode carrying the positive charge) inserted
in the receiver compartment and the cathode
(electrode carrying the negative charge) in the

10   donor compartment. The concentration of calcein in
the donor and the receiver compartment was measured
using the spectrofluorimeter (Photon Technology
Int). The concentration of glucose in the donor
and the receiver compartment was measured using

15   scintillation counter.
Results:
    a)   **Transdermal Calcein Transport:**
        Calcein possesses a molecular weight of 622
and a net charge of -4. Due to its charge and

20   relatively large size, passive transdermal
transport of calcein is extremely low. Figure 9
shows the transdermal transport of calcein during
sonophoresis, iontophoresis or combination thereof.
Sonophoresis alone at 20 KHz and 125 mW/cm^2 for 10

25   minutes followed by a waiting period of 1 hour
(total time of 70 minutes) resulted in transdermal
transport of about 3.2 x 10^{-3} % of calcein present
in the donor compartment. Similarly, application
of electric current (0.2 mA/cm^2) alone for 70

30   minutes induced transdermal transport 2.5 x 10^{-3} %
of calcein present in the donor compartment. A
combination of the two methods: 10 minutes of
sonophoresis (20 KHz, 125 mW/cm^2) and 70 minutes of
simultaneous iontophoresis (0.2 mA/cm^2) (ultrasound

35   and electric current ON for the first 10 minutes
with only iontophoresis ON for the next 60 minutes)
resulted in transdermal transport of about 3.5 x 10$^-$

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¹% of calcein present in the donor compartment. The
results show that transdermal calcein transport
during a combined treatment of sonophoresis and
iontophoresis is about 100-fold higher than that
5   during sonophoresis or iontophoresis under similar
conditions.

      b) **Transdermal Glucose Extraction:**

      Glucose is a hydrophilic molecule and shows no
detectable transdermal transport passive diffusion.
10   Figure 10 shows the amount of glucose extracted
transdermally by sonophoresis, iontophoresis or
combination thereof. Application of ultrasound (20
KHz, 125 mW/cm²) alone for 10 minutes followed by a
waiting period of 1 hour (total time of 70 minutes)
15   resulted in the donor glucose concentration of
about 1% of the receiver glucose concentration.
Application of electric current (0.2 mA/cm²)
(ultrasound and electric current ON for the first
10 minutes with only iontophoresis ON for the next
20   60 minutes) resulted in a donor glucose
concentration which was about 3.4% of the receiver
concentration. Simultaneous application of
sonophoresis and iontophoresis induced about three-
fold higher transdermal glucose transport than that
25   induced by sonophoresis alone.

EXAMPLE 6:   **Comparison of drug transfer through
skin using ultrasound or electrical
field alone or in combination.**

      MATERIALS AND METHODS

30   **A.   Materials**

      Full thickness of human cadaver skin (obtained
from local hospitals) was heat stripped by
immersion in 60°C water for two minutes followed by
the removal of the epidermis. The skin was then
35   stored in a humidified chamber (95% relative
humidity) at 4°C. The heat-stripped human
epidermis was placed in a custom-made side-by-side
permeation chamber, skin area of 0.64 cm², designed

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to adapt an ultrasound transducer at the donor
side. The donor compartment was filled with a 1 mM
solution of calcein (MW 623, electric charge - 4;
Sigma Chemicals) (CA) and 1 mM sulphorhodamine (MW

5   607, electric charge - 1; Sigma Chemical) (SR) in
150 mM Phosphate Buffer Saline (PBS; Sigma
Chemicals).

    The ultrasound probe was inserted into the
donor compartment. The direction of the ultrasound

10 wave was perpendicular to the membrane surface.
The stratum corneum was facing the donor
compartment. Both donor and receptor compartments
were filled with degassed phosphate buffer saline
(PBS) pH=7.4. The temperature was followed to be

15 in the range of 22 ± 2°C. SR and CA were added to
the donor compartment to provide concentration of 1
mM CA and 1 mM SR. Fresh PBS was continuously
pumped into the receptor compartment at 0.8 ml/min
from a reservoir.

20   **B.   Fluorescence measurements**
    The fluorometer was set up for dual wavelength
measurements (excitation wavelength = 488 nm,
emission wavelength = 515 nm (calcein), and
excitation wavelength = 586 nm, emission wavelength

25 = 607 nm (sulphorhodamine)). The sample cuvette of
the fluorometer was sealed but for two openings
that were provided for the flow of receiver fluid
through it. A small custom-made electric stirrer
was installed in the cuvette so that there were no

30 stagnant zones in it. Care was taken to avoid any
obstruction of the excitation beam by the stirrer.
Transdermal calcein and sulphorhodamine flux was
calculated from the fluorescence readings by taking
into account parameters such as flow rate, receiver

35 compartment volume, and fluorometer caveat volume.
The effluent from the receptor compartment was
pumped through a spectrofluorometer (Fluorolog-II-

system F112AI SPEX-industries, Edison, NJ) where
the fluorescence of calcein and sulphorhodamine was
separately measured twice every minute. The
excitation for CA is 488 nm and for SR 586 nm, the
measurement for CA was at 515 nm and for SR 607 nm.
The receptor was mixed by an electromechanical
stirrer. The fluorescence measurements were
deconvoluted to calculate the CA and SR flux.

C.   **Application of Ultrasound**

Two studies were conducted. In the first,
two ultrasound sources were utilized:

i.   20 KHz Sonics and Materials (250 W) with a
probe surface area of 0.25 cm^2.

ii.   1 MHz Sonopuls Therapeutic device with
probe surface area of 0.8 cm^2. Pulsed and
continuous modes were evaluated below 2 W/cm^2 for
the continuous mode and 2-3 W/cm^2 pulsed (20% duty-
cycle). The distance of the probe tips from the
skin was 3 cm for the 20 KHz and 4 cm for the 1
MHz.

In the second study, ultrasound was applied
under therapeutically approved conditions (1.4
W/cm^2, 1 MHz and 3 MHz, continuous) using a
sonicator (Sonopuls 463, Henley International) for
various exposure times up to 1 hour. The
ultrasound transducer was located at a distance of
about 3 cm from the skin.

D.   **Electroporation**

One Ag/AgCl electrode (In vivo metric,
Healdsburg, CA) was located in the donor and one in
the receptor compartment, so that the distance of
electrodes from the skin was equal in both the
compartments (about 8 mm). Voltage pulses were
applied using a pulse generator (ECM 600, BTX, San
Diego, CA) across the electrodes such that the
positive electrode was always in the receptor
compartment. This provided an electric driving

force for calcein and sulphorhodamine (both negatively charged) to transport across the skin. The voltage applied to the electrodes divides between the saline and the skin. The voltage drop

5 across the skin was estimated using the measured electrical resistance of the skin and saline. The magnitude as well as the length of the voltage pulses was varied over a wide range in order to investigate their effect on transdermal transport.

10 In the first set of experiments (Figures 11a, 11b, 11c), a voltage divider of 10:40 ohm was used to provide a fixed time constant (exponential shape pulse). The maximum pulsing voltage in all experiments was 750 volts across the chamber

15 (refers to a voltage drop across the skin of 210 - 230 volts). The pulse rate was 1 pulse/min for 60 minutes, controlled by a computer.

In the second set of studies (Figures 11c, 12b and 12c, 13), the electric field (100 V) was

20 applied across the skin, exponentially decaying pulse with a time constant (τ) of 1 millisecond, one pulse applied every minute.

In order to assess the stability of these molecules during electroporation, calcein and

25 sulphorhodamine solutions (1 mM each) were exposed to electroporating conditions similar to those used in this study. No difference between the intensity of their fluorescence before and after exposure to electric fields could be detected. In addition,

30 these molecules are stable up to a temperature of 100°C (measured in terms of fluorescence). When these molecules are degraded, they do not fluoresce. In general, these molecules have been found to be very stable against many physico-

35 chemical changes.

E.   Measurements of Passive Electric Skin
       Properties

A second pair of electrodes (same type as
above) was used for monitoring the passive
5   electrical properties (specifically, electrical
resistance). Since the electrical resistance of
the skin is a good indicator of its barrier
properties, the skin resistance was measured
before, during and after the experiments. The
10   effect of electroporation and ultrasound separately
and together on skin electrical resistance was
determined. If the electrical resistivity before
the application of either ultrasound or
electroporation was lower than 20 kΩ-cm^2 or if any
15   significant passive calcein or sulphorhodamine
transdermal flux was observed (that is, J greater
than 0.002 μg/cm^2/h (the detection limit)), the skin
piece was considered leaky and replaced by a new
piece.

20   RESULTS AND DISCUSSION

A.   Application of Ultrasound Enhances the
       Efficacy of Electric Field.

The results of the first study are shown in
Figure 11a. Figure 11a shows the time variation
25   flux of SR which permeated the skin with time.
After 400 sec of passive diffusion, pulsed
ultrasound (1 MHz, 20% duty cycle, 2.5 - 2.9 W/cm^2)
was turned on for 2750 sec. The ultrasound was
turned off at 2750 sec. High voltage pulsing was
30   turned on at 6900 sec for 1 hour (10,500 sec end of
electroporation pulsing). Ultrasound (1 MHz, 0.8
cm^2 20% duty cycle, 2.5 - 2.9 W/cm^2) was turned on
again at 14,310 sec, electroporation (same
condition) was turned on again at 15,200 sec while
35   the pulsed ultrasound was on. At 16,440 sec the
ultrasound wave was changed from pulsed to
continuous while the electroporation continued.

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The experiment was terminated at 20,040 sec. The
experimental procedure is summarized in Table 5
below.

TABLE 5:   Conditions used for determining effect of
ultrasound and electroporation

From (sec)	To (sec)	Description of the transdermal transport
0	400	passive diffusion
400	3150	pulsed ultrasound
3150	6900	passive diffusion
6900	10500	electroporation
10500	14310	passive diffusion
14310-	15200	pulsed ultrasound
15200-	16400	electroporation + pulsed ultrasound
16440-	20040	electroporation + continuous ultrasound

Figure 11b and 11c show the effect of
simultaneous application of ultrasound (1 MHz, 1.4
W/cm^2, continuous application) and electric field
(100 V across the skin, exponentially decaying
pulse with a time constant (τ) of 1 millisecond,
one pulse applied every minute) on the transdermal
transport of calcein and sulphorhodamine
respectively. The passive transdermal transport
(in the absence of ultrasound and electric field)
is below the detection limit and is not shown in
Figure 11b or 11c. Application of ultrasound alone
does not enhance the flux of calcein or
sulphorhodamine. However, application of
ultrasound enhanced steady-state transdermal flux

of both calcein and sulphorhodamine during electric
field pulsing. The enhancement is quantitatively
defined as the amount of calcein or sulphorhodamine
transported in the presence of ultrasound-electric

5   field pulsing to that in the presence of electric
field pulsing alone. This ratio is 2 in the case
of calcein (Figure 11b), and 3 in the case of
sulphorhodamine (Figure 11c). Application of
ultrasound also reduced transdermal calcein

10   transport lag time, defined as the time required to
reach the steady state, from a typical value of 15
minutes in the presence of electric field alone to
about 9 minutes in the presence of ultrasound and
electric field.

15   Similar effects of ultrasound on transdermal
transport of SR and CA during electroporation can
be also seen in Figures 12a and 12b which present
the flux of CA (Figure 12a) and SR (Figure 12b) in
experiments where the skin samples were exposed

20   continuously to electroporation and continuous
ultrasound (1 MHz, 0.8 cm^2, 2 W/cm^2) (o) and
controls (x) where the skin was exposed to electric
fields alone. The possible mechanism for this
phenomena might be that the electrical pulsing

25   creates short term pores in the skin while
ultrasound is forcing the solutes through these
pores.
     In order to quantitatively estimate the
reduction in the required pulsing voltages by

30   simultaneous application of ultrasound and electric
field, transdermal sulphorhodamine transport was
measured in the presence as well as absence of
ultrasound (1 MHz, 1.4 W/cm^2) and electric field
(voltage across the skin increased from 20 V to 150

35   V in steps of 5 V every 30 minutes, 1 millisecond
exponential pulse applied every minute).

Figure 13 shows the variation of transdermal
sulphorhodamine flux with voltage across the skin
in the presence (O) as well as in the absence (X)
of ultrasound. The transdermal sulphorhodamine
5   flux is nearly zero as long as the voltage is below
the threshold value and thereafter increases
linearly with voltage. The threshold voltage for
this pulsing protocol can be estimated by measuring
the intercept of the linear variation of flux with
10   voltage on the voltage axis. In the absence of
ultrasound, this threshold is about 53 ± 3 V and
that in the presence of ultrasound is about 46 ± 3
V, indicating that application of ultrasound
slightly reduces the threshold pulsing voltage.
15   Figure 13 also shows that the transdermal
sulphorhodamine flux at various pulsing voltages is
always higher in the presence of ultrasound. Thus,
the pulsing voltage required to achieve a given
transdermal flux is smaller in the presence of
20   ultrasound. For example, to achieve a transdermal
sulphorhodamine flux of 0.15 μg/cm^2/hr, the required
voltage is about 95 V in the absence of ultrasound
and 75 V in the presence of ultrasound.
Cavitation may play a two-fold role in
25   enhancing the effect of electric field on
transdermal transport. Since the electrical
resistance of the disordered bilayers is likely to
be smaller than that of the normal lipid bilayers,
the applied electric field may concentrate
30   preferentially across the normal bilayers. This
may decrease the threshold electroporating voltage
for transdermal transport of calcein and
sulphorhodamine. Application of ultrasound reduces
the threshold pulsing voltage from about 53 ± 3 V
35   in the absence of ultrasound to about 46 ± 3 V in
the presence of ultrasound (a reduction of about
12%). This number is comparable to an independent

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estimate of the fraction of SC bilayer disordered by ultrasound application (15%).

The oscillations of cavitation bubbles may also induce convection across the skin. In order
5   to assess the role of convection in the synergistic effect of ultrasound and electric field, transdermal calcein and sulphorhodamine transport was measured sequentially in the presence of electric field alone, ultrasound and electric
10 field, ultrasound alone and in the absence of ultrasound and electric field. The results of these sequential procedure are shown in Figure 14. Results from a single experiment are shown to depict the shape of the curves clearly. Note the
15 change in the transdermal flux at 1 and 1.5 hours when electric field and ultrasound is turned OFF respectively. If electrophoresis plays an important role in calcein and sulphorhodamine transport, the transdermal flux is likely to
20 decrease rapidly after *electric fields* is turned OFF. On the other hand, if cavitation-induced convection plays an important role, transdermal flux would rapidly decrease after turning *ultrasound* OFF. Indeed, calcein flux decreases
25 rapidly after turning electric field OFF (1 hour) and achieves a value comparable to the background flux. When ultrasound is turned OFF at 1.5 hours, calcein flux further decreases by a small amount (compared to the reduction after turning electric
30 field OFF at 1 hour) and thereafter it remains nearly at the background level. This suggests that calcein transport is mainly driven by electric forces. On the other hand, convection appears to play an important role in transdermal
35 sulphorhodamine transport in the presence of ultrasound and electric field because the sulphorhodamine flux *did not decrease rapidly* after

turning electric fields OFF, but decreased
instantaneously after turning ultrasound OFF at 1.5
hours. The total decrease in the transdermal
sulphorhodamine flux after turning the electric

5   field OFF (that is, between a period of 1 and 1.5
hours) is comparable to the instantaneous decrease
in its value after turning ultrasound OFF at 1.5
hours. This suggests that both electric field and
ultrasound-generated convection may play an

10 important role in transdermal sulphorhodamine
transport. This difference in the behavior of
calcein and sulphorhodamine is presumably because
calcein possesses a much larger charge (-4)
compared to sulphorhodamine (-1). In this respect,

15 it is important to note that the transdermal
transport of calcein and sulphorhodamine in the
presence of electric field alone also differs
significantly. Calcein transport increases rapidly
and achieves a steady state within 15 minutes.

20 Sulphorhodamine flux, however, increases
continuously with time over the experimental
duration. This difference in the behavior of
calcein and sulphorhodamine flux may also be
attributed to the lower charge on sulphorhodamine,

25 as the transport during the electrical pulses is
driven by electrophoresis.

The combined effect of electroporation and
ultrasound on transdermal flux in all experiments
was higher for SR than CA, suggesting that the

30 additional enhancement by ultrasound is more
effective on less charged molecules. The effect of
ultrasound was observed on both the lag time and
the steady state flux for the two molecules.

In summary, electroporation of the skin

35 resulted in a very significant increase in SR
permeability. The phenomenon was observed also on
repeated application of electroporation, but the

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enhancing effect was only slightly higher.
Application of ultrasound without electroporation
did not result in enhanced flux. The very
pronounced increase in permeability was observed

5   when the skin was exposed to the combined effect of
ultrasound and electroporation (more than twice the
flux value observed with electroporation without
ultrasound). The combined effect of ultrasound and
electroporation was also observed in additional

10   exposures of the same skin specimens.

EXAMPLE 7:   Determination of effect of
**   ultrasound on skin.**

The following experiment was measured in order
to assess whether application of ultrasound induces

15   any irreversible change in the skin structure.
Human skin pieces were exposed to electric field
alone (100 V across the skin, exponentially
decaying pulse with a time constant (τ) of 1
millisecond, one pulse applied every minute), then

20   simultaneously to ultrasound (1 MHz, 1.4 W/cm^2)-
electric field and again to electric field alone.
A comparison of sulphorhodamine transport due to
the electric field alone, before and after the
simultaneous electric field-ultrasound treatment,

25   indicated that the flux returns to a near baseline
value, suggesting that the application of
ultrasound did not induce any irreversible
alteration in the barrier properties of skin. The
recovery was also supported by electric resistance

30   measurements indicating that application of
ultrasound did not cause any irreversible change in
the electrical resistance of the skin.

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We claim:

1.   An apparatus for enhancing transport of molecules across the skin comprising means for applying ultrasound to the skin at a frequency of between 20 kHz and less than 1 MHz for a period of time effective to transport the molecules.

2.   The apparatus of claim 1 wherein the molecules are drugs to be administered to a person in need thereof.

3.   The apparatus of claim 1 wherein the molecules are analytes present in blood, lymph or interstitial fluid.

4.   The apparatus of claim 1 wherein the frequency is between 20 and 45 kHz.

5.   The apparatus of claim 1 wherein the intensity is between zero and 1 W/cm^2.

6.   The apparatus of claim 5 wherein the intensity is between 12.5 mW/cm^2 and 225 mW/cm^2.

7.   The method of using the apparatus of any of claims 1-6 to enhance transfer of molecules through the skin.

8.   A method for collecting an analyte to be measured in a blood or lymph sample comprising applying at an appropriate site for collection of a sample an effective amount of ultrasound and collecting the sample, wherein the ultrasound is applied at a frequency between 20 kHz and 10 MHz.

9.   An apparatus for enhancing transdermal transport of molecules comprising means for administering to the skin an effective amount of ultrasound in combination with an enhancer selected from the group consisting of chemical enhancer combinations of agents enhancing solubility of the molecules to be transported with agents enhancing the fluidity of lipid bilayers, mechanical force fields, osmotic force fields, magnetic force fields and electric force fields.

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10.   The apparatus of claim 9 wherein the ultrasound is administered at a frequency of less than 2.5 MHz.

11.   The apparatus of claim 9 wherein the ultrasound is administered at a frequency of 1 MHz or less.

12.   The apparatus of claim 9 wherein the combination is linoleic acid in an ethanol solution.

13.   The apparatus of claim 9 wherein the ultrasound is administered in combination with an electric force field selected from the group consisting of electroporation and iontophoresis.

14.   The apparatus of claim 13 wherein the electric field is pulsed.

15.   The apparatus of claim 9 wherein the ultrasound is administered in combination with a magnetic force field.

16.   The apparatus of claim 9 wherein the ultrasound is administered in combination with a mechanical force field created by pressure or vacuum.

17.   The apparatus of claim 9 wherein the ultrasound is administered in combination with an osmotic force field.

18.   The apparatus of claim 9 wherein the ultrasound is administered in combination with chemical enhancers and mechanical forces.

19.   The apparatus of claim 1 wherein the molecules to be transported are drugs the patient is in need of.

20.   The apparatus of claim 9 wherein the compound to be transported is an analyte to be measured.

21.   The apparatus of claim 9 wherein the ultrasound is pulsed.

22. The method of use of the apparatus of any of claims 9-21 in enhancing transport of molecules through the skin.

22. A composition for enhancing delivery of drugs across the skin comprising drug encapsulated in a liposome or polymeric carrier for application to the skin and applying ultrasound for a period of time effective to deliver to the patient a desired drug dosage.

23. The composition of claim 22 wherein the microparticle is coated with a lipophilic or hydrophilic material enhancing transdermal penetration.

24. The composition of claim 22 wherein the material is a hydrophilic molecule couple to a synthetic biodegradable polymer forming the microparticle.
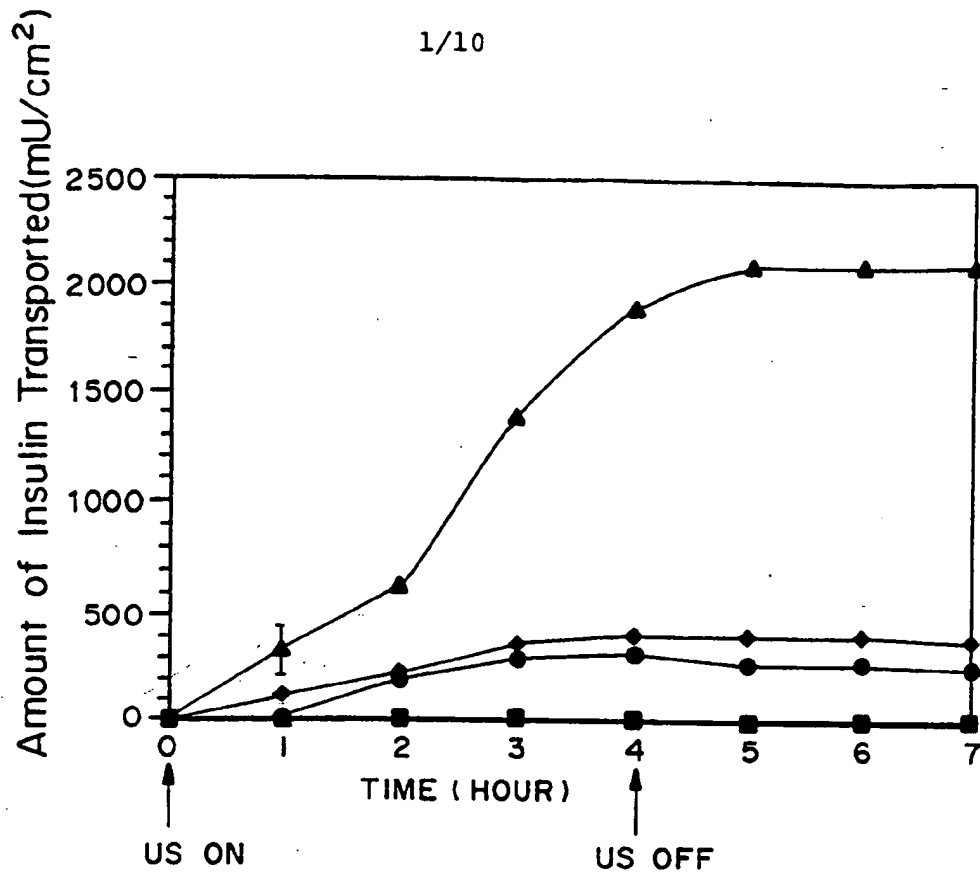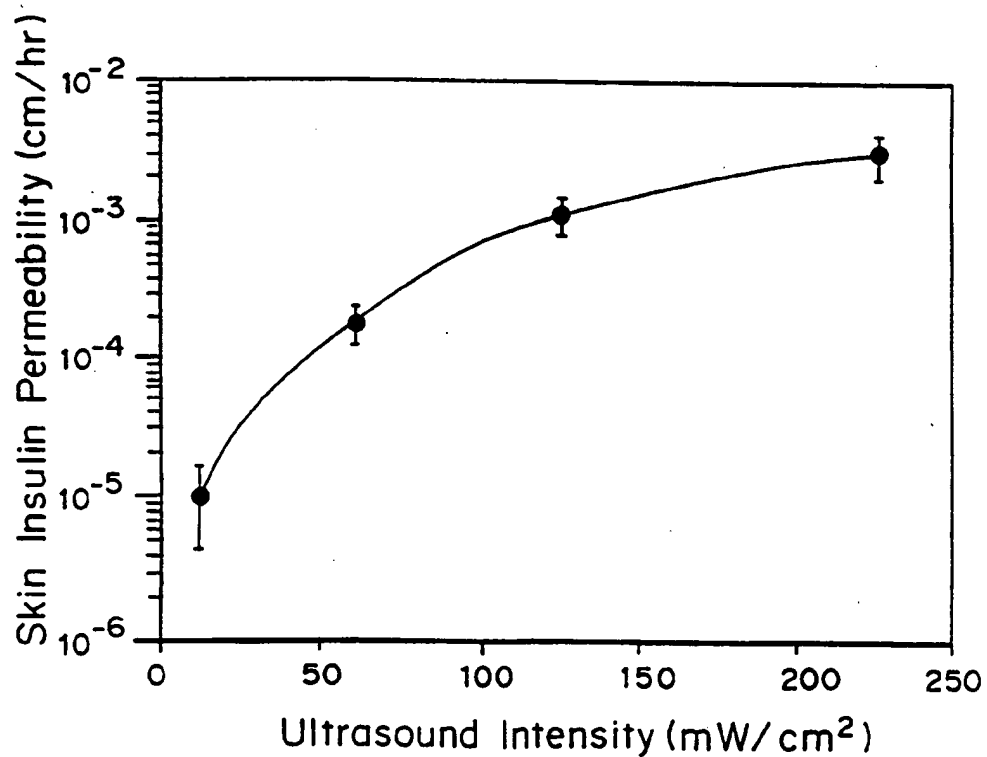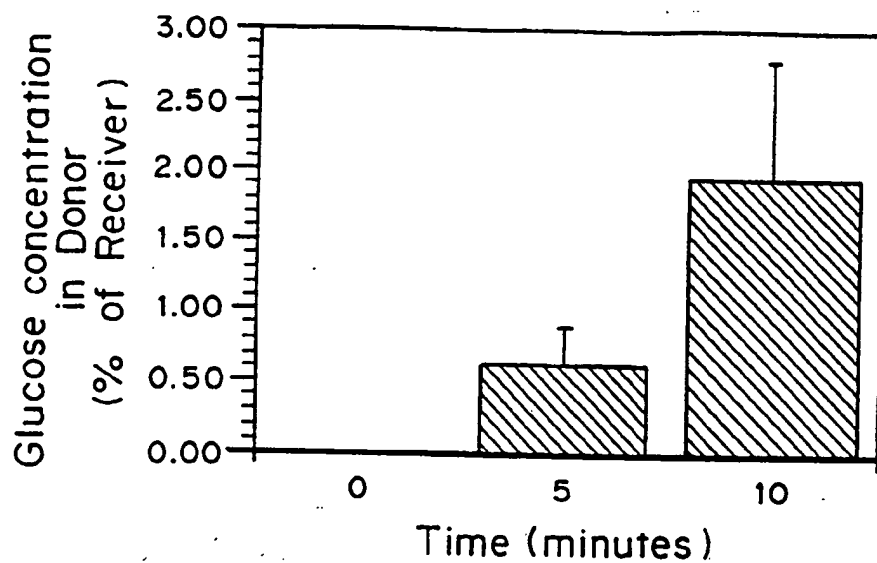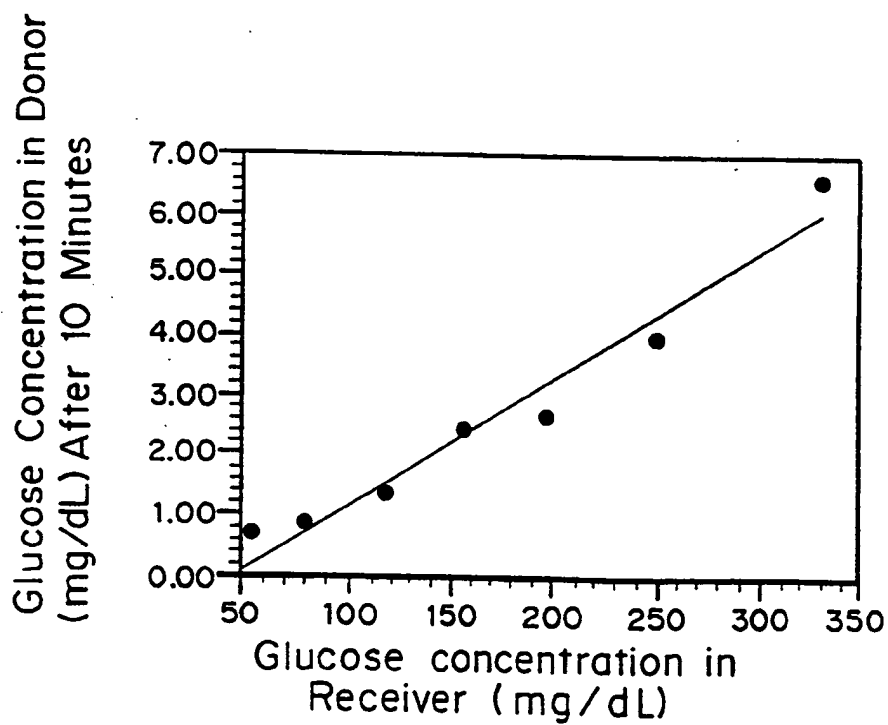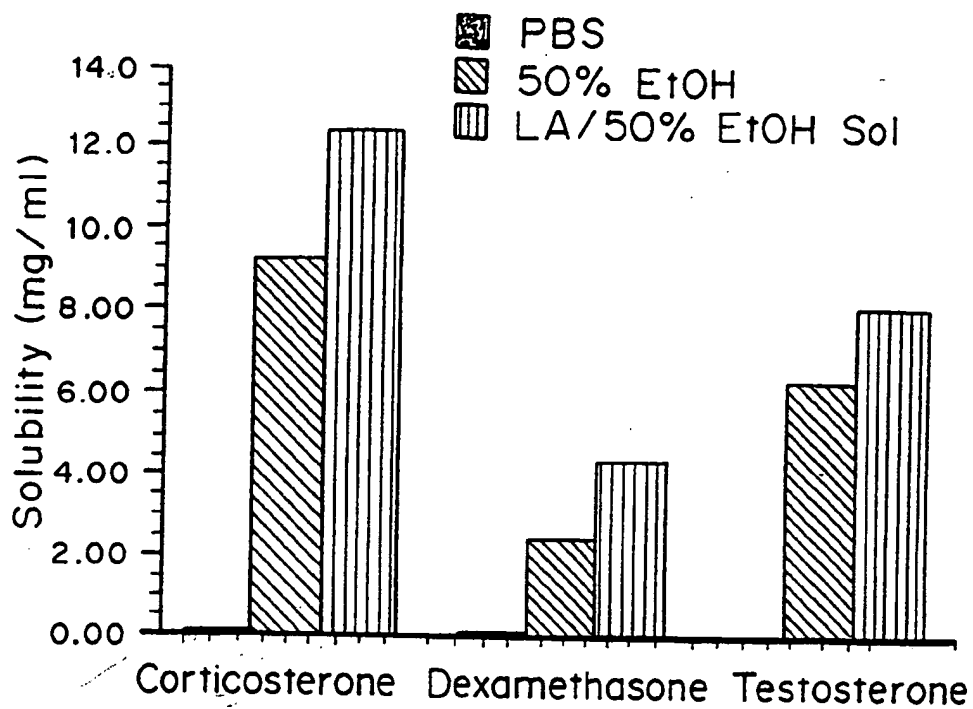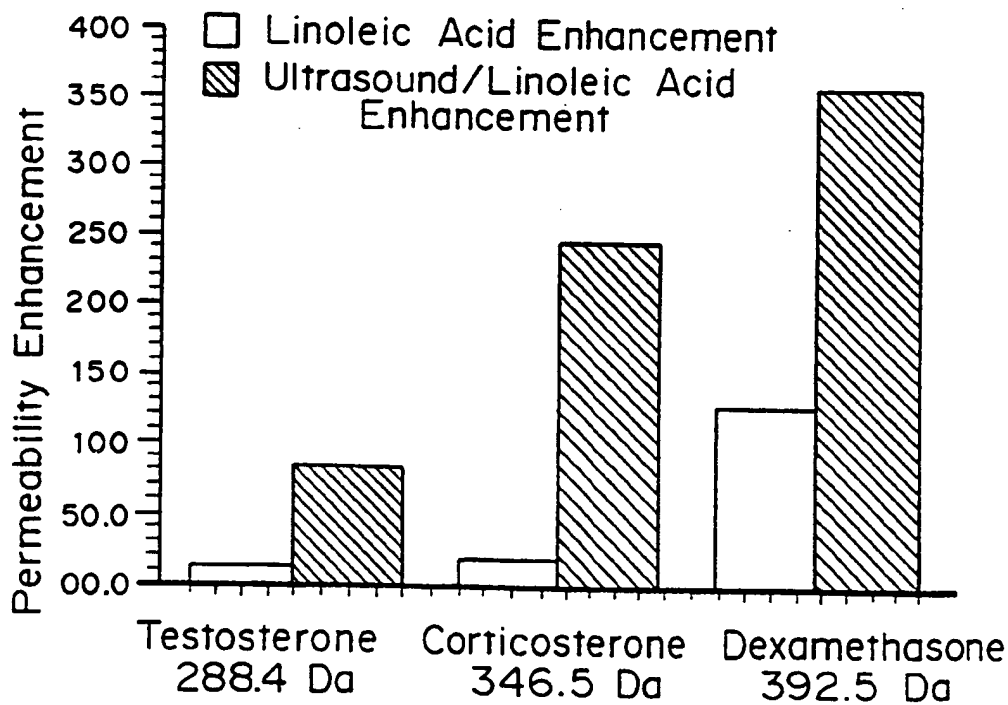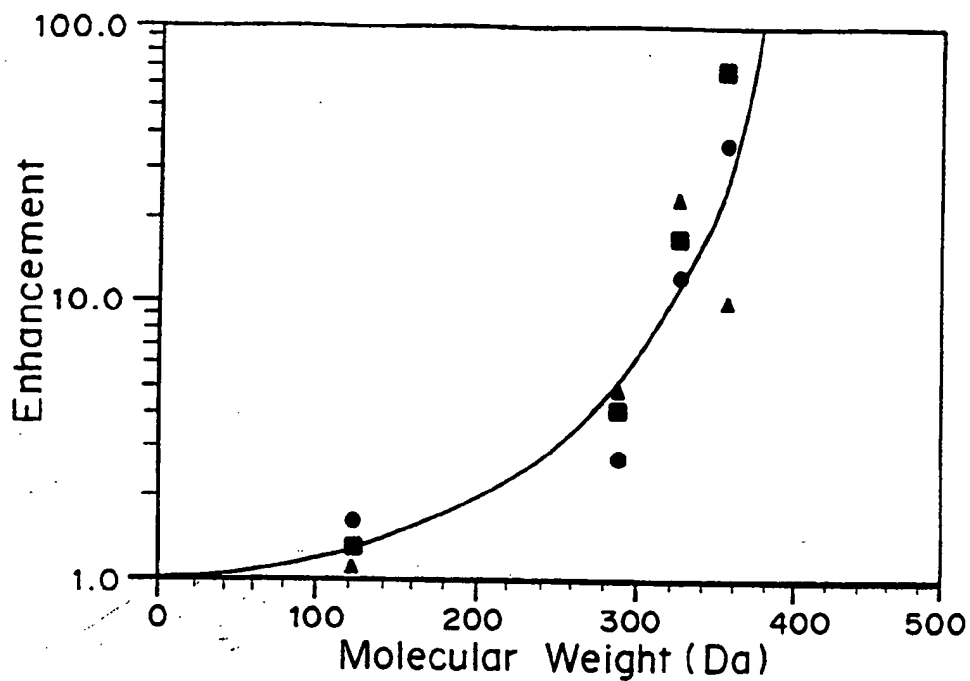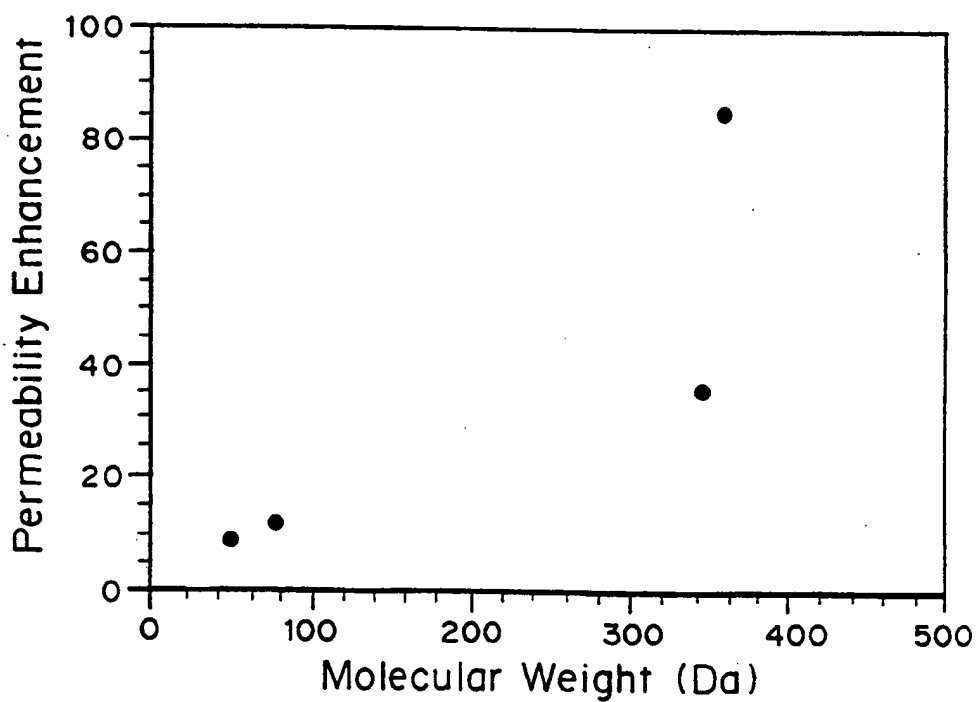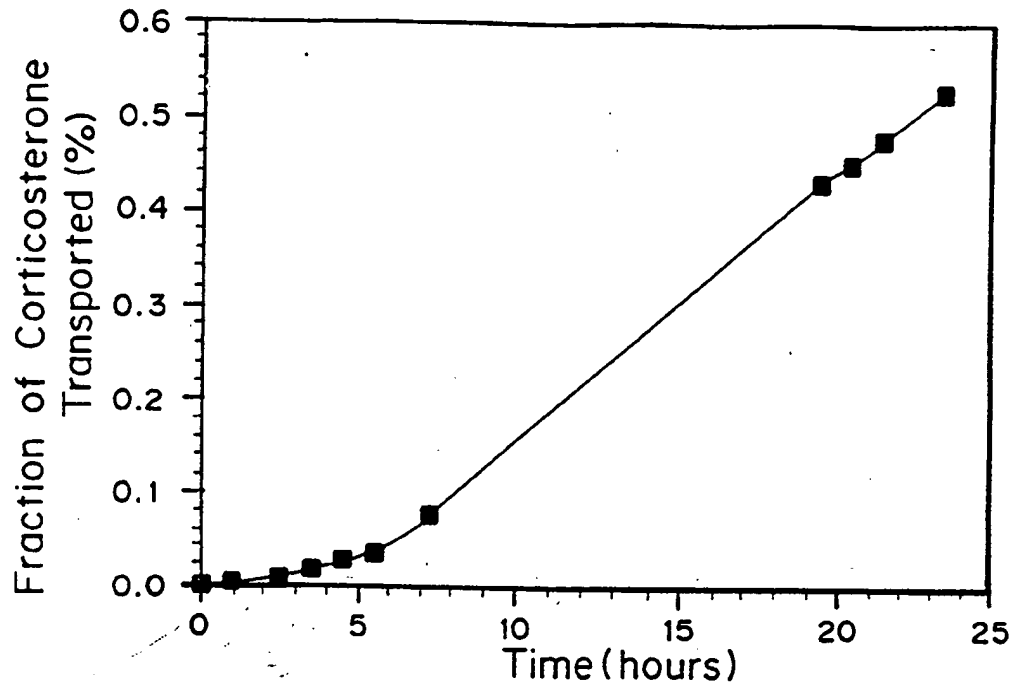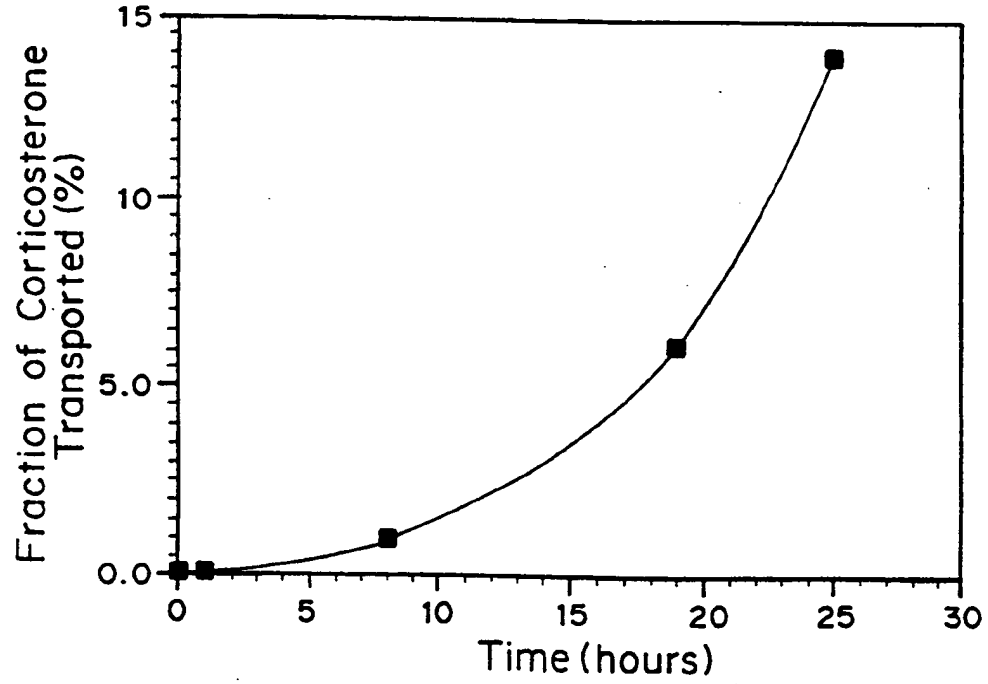
FIG. 1a

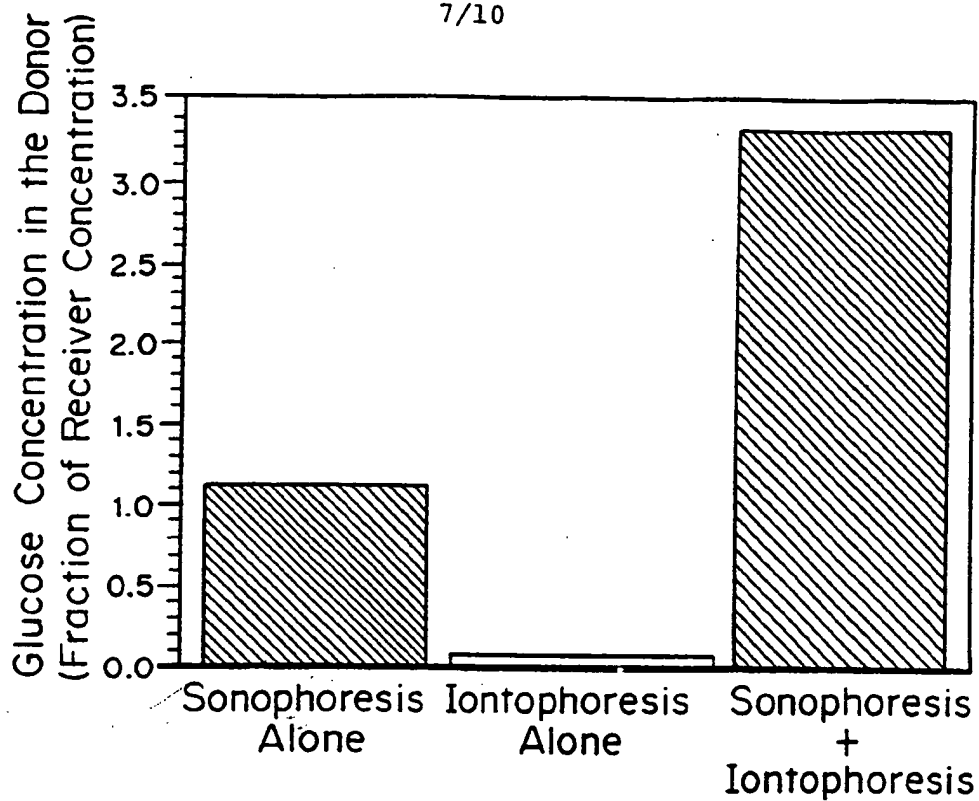FIG. 1b

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6a

FIG. 6b

FIG. 7a

FIG. 7b

FIG. 8

FIG. 9

FIG. 10

FIG. 11a

FIG. 11b

FIG. 11c

flux with and without us-exposure (1 MHz)

FIG. 12a

FIG. 12b
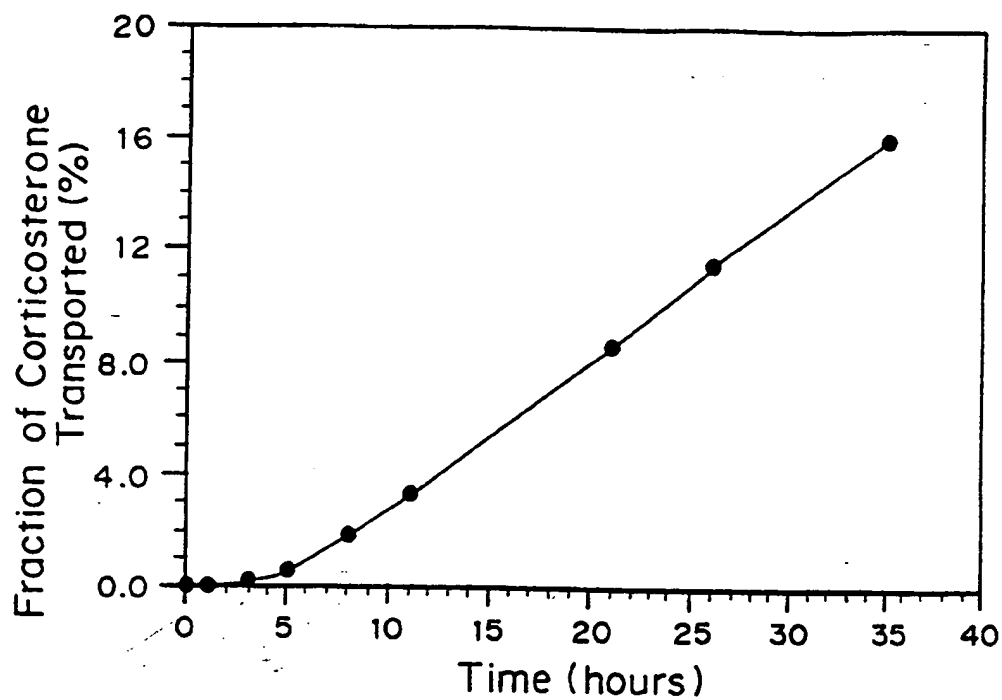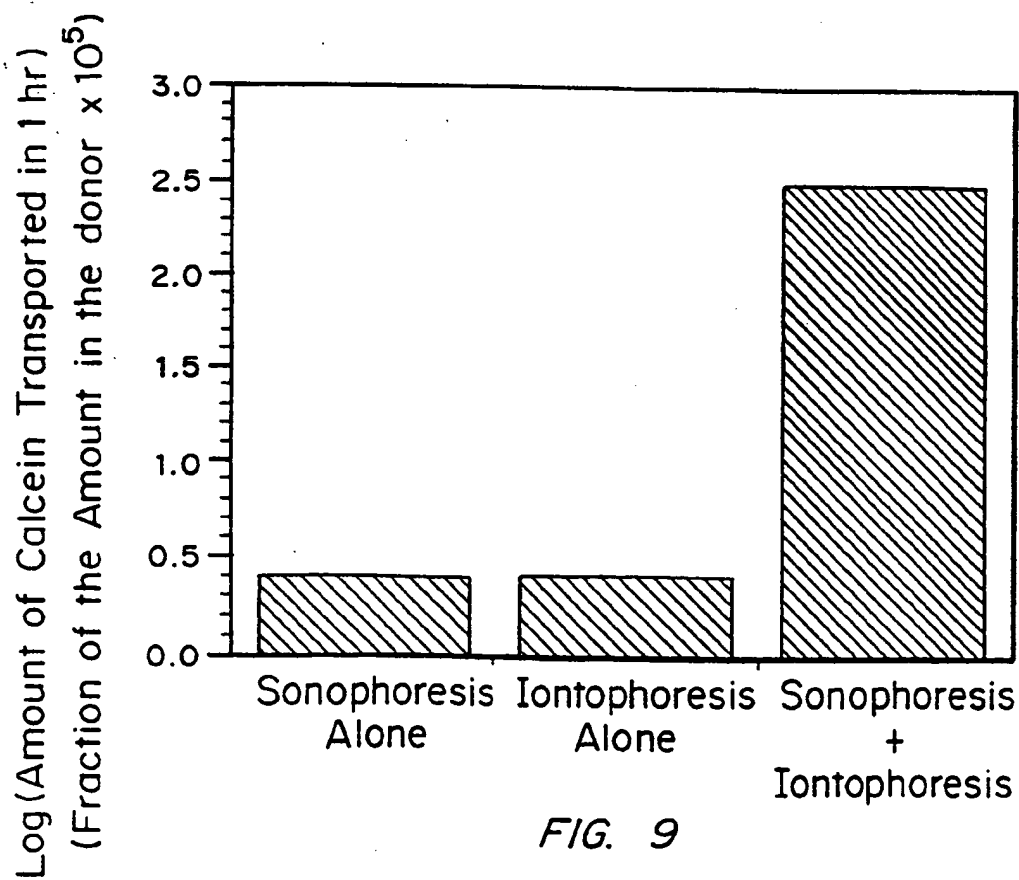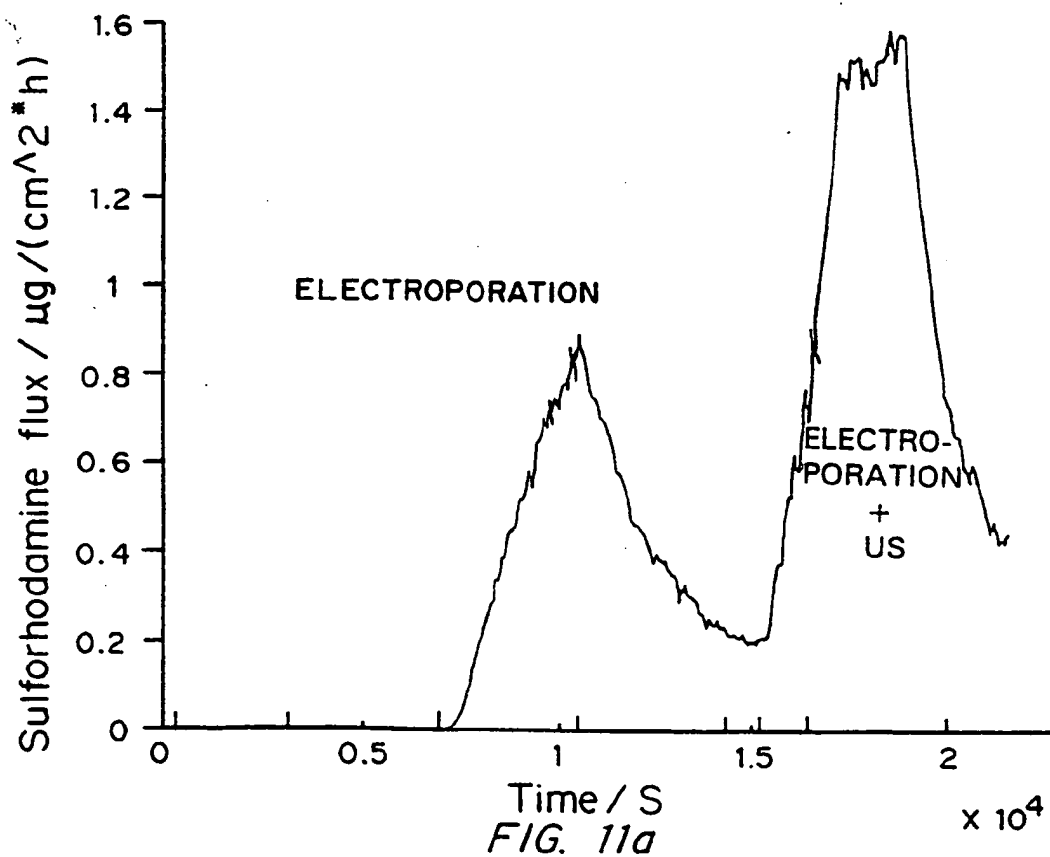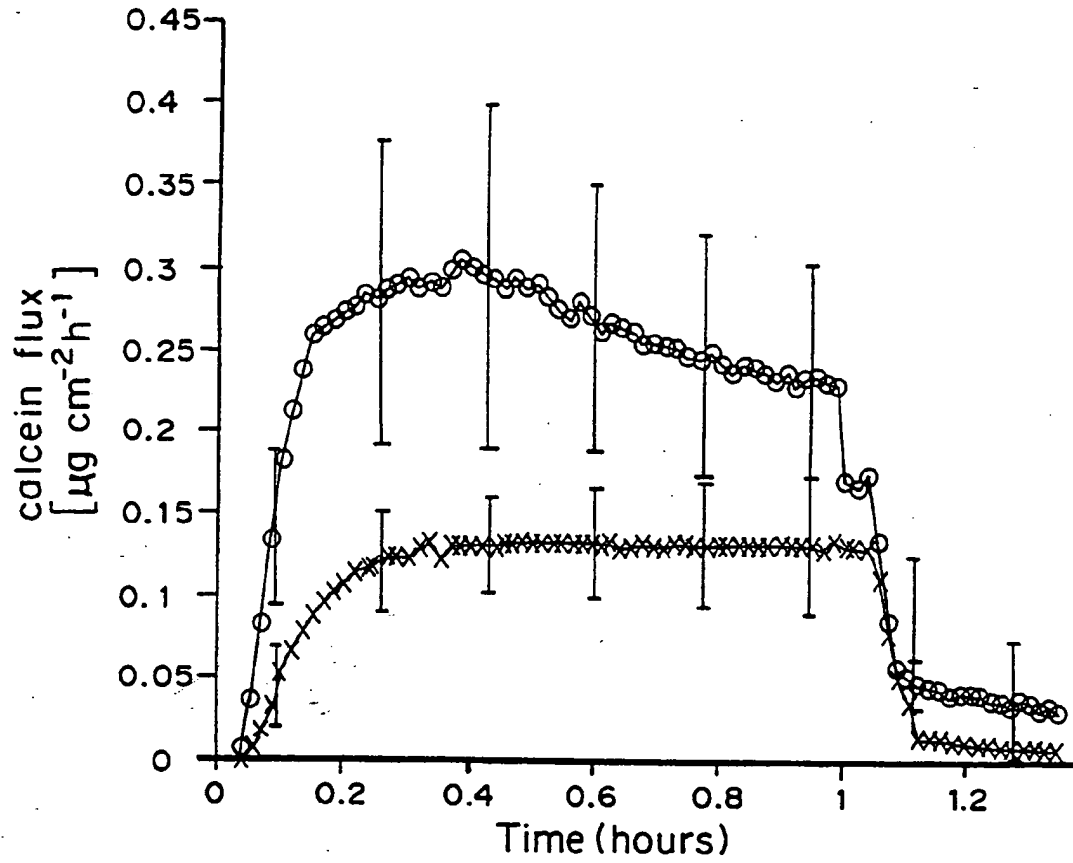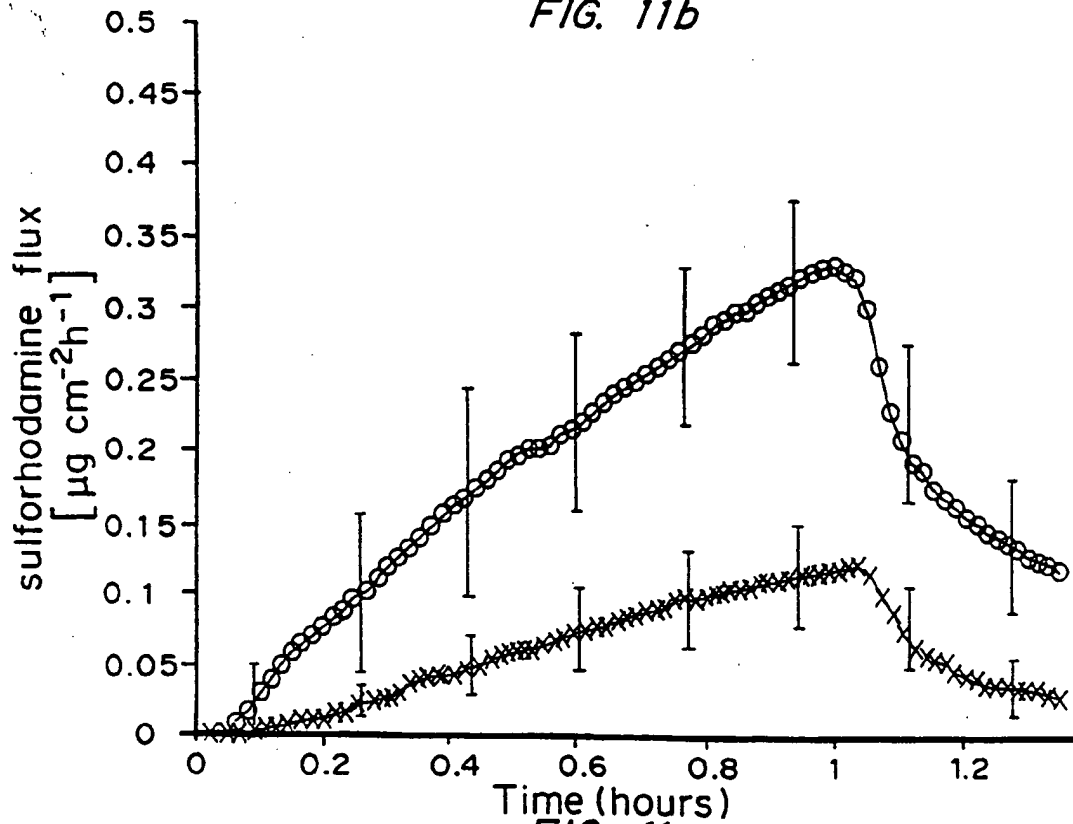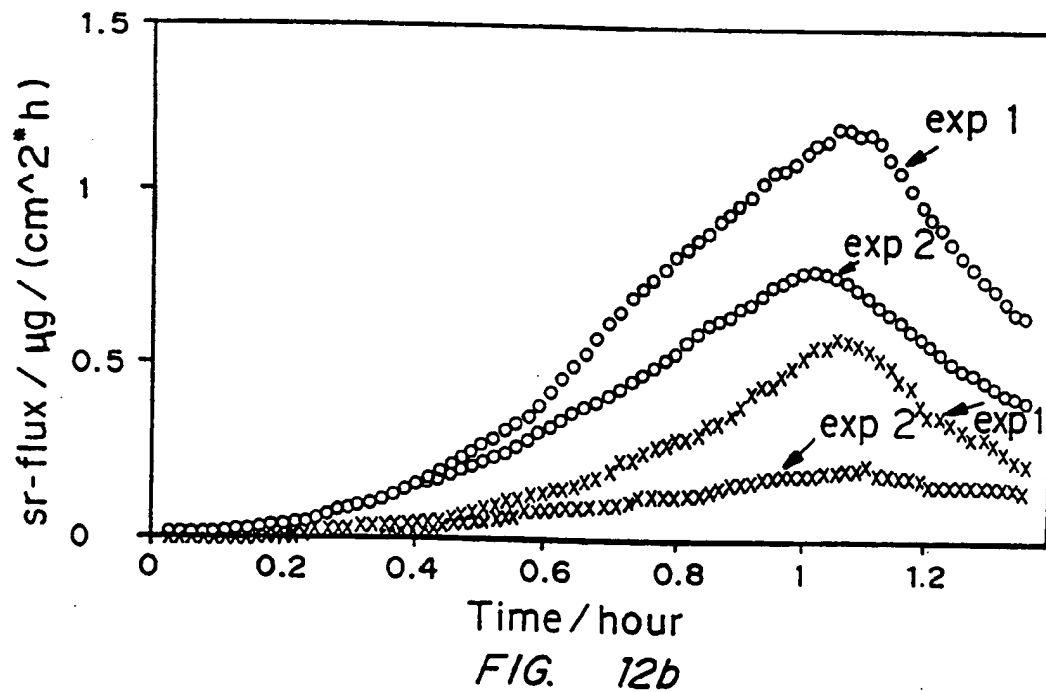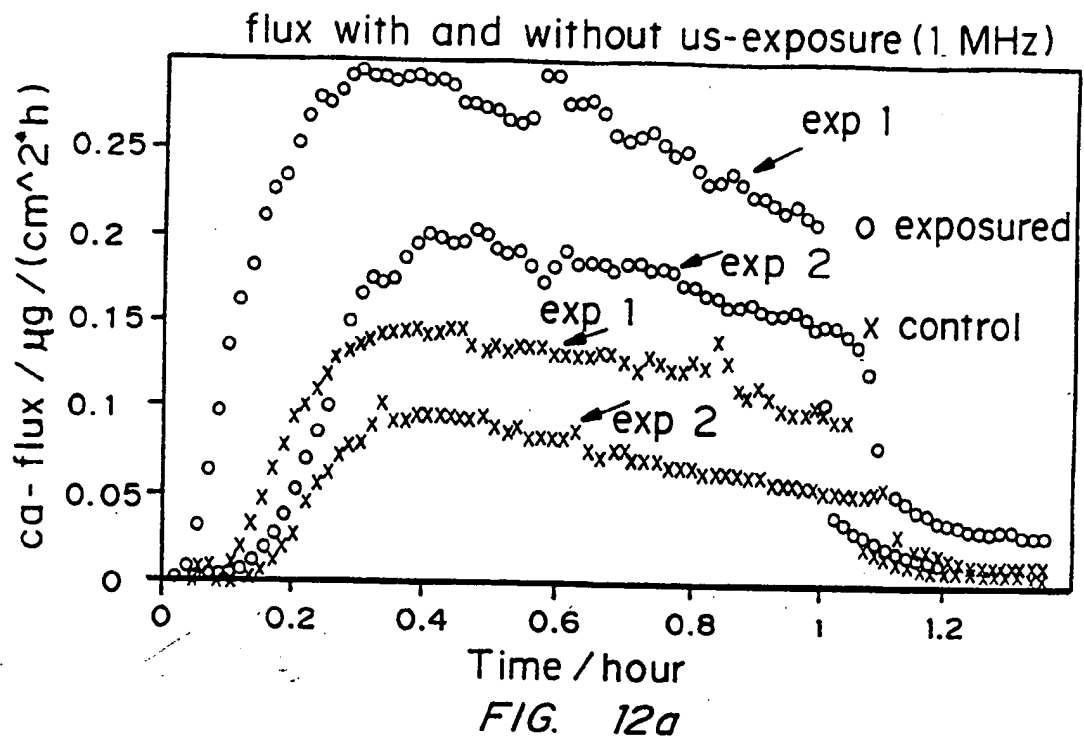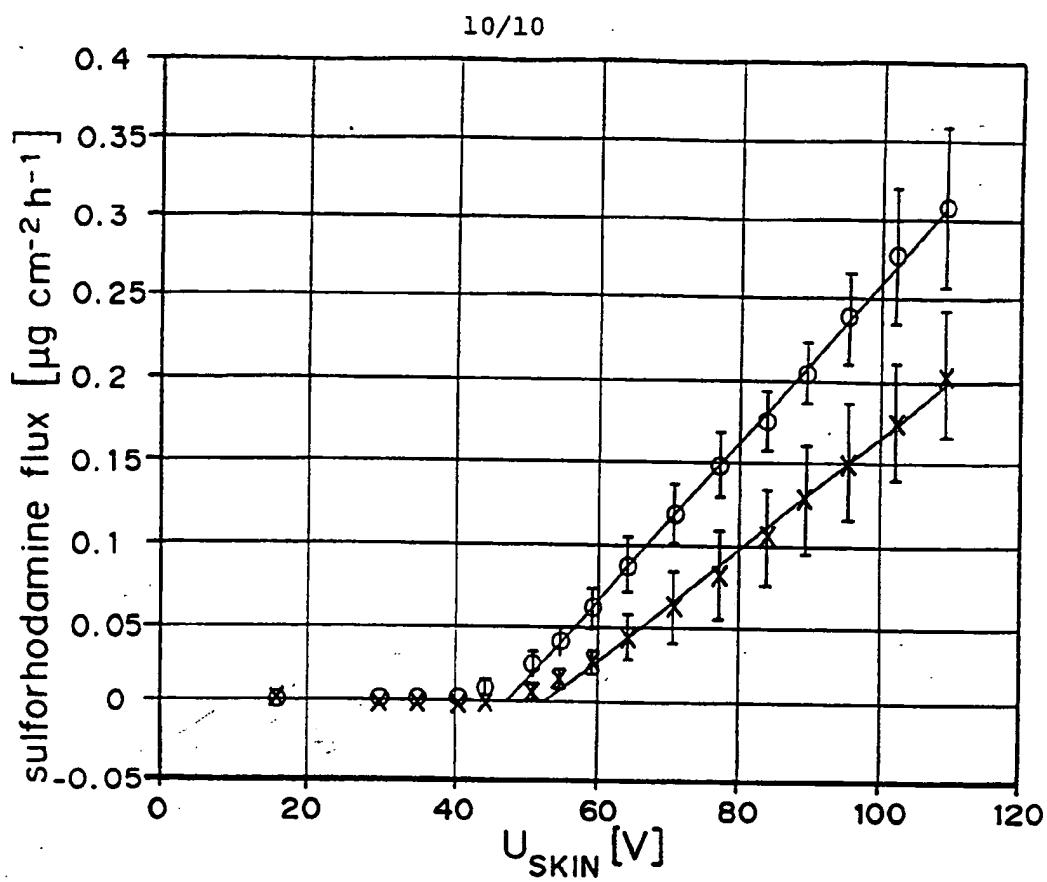
FIG. 13
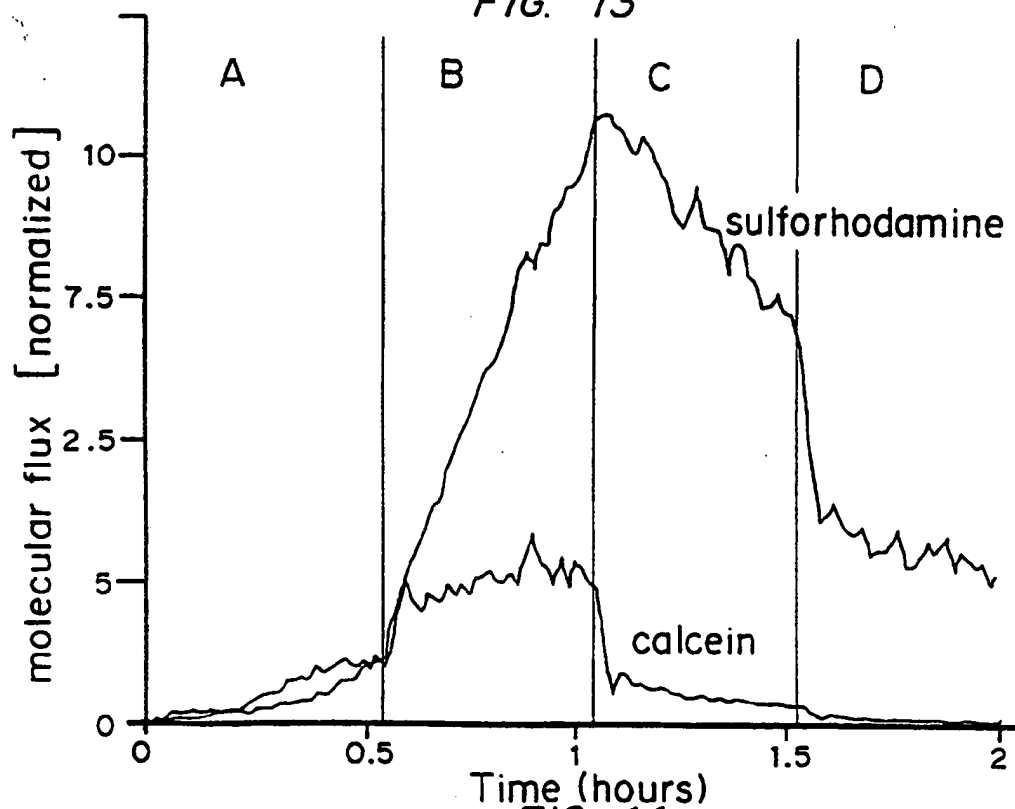
FIG. 14

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61M37/00   A61N1/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61M   A61N   A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,88 00001 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 14 January 1988 see page 1, line 1 - line 3 see page 2, paragraph 2 - page 3, paragraph 1 see page 5, paragraph 6 - page 6, paragraph 2 see page 8, paragraph 2 - paragraph 3 see page 10, paragraph 3 - page 11, paragraph 3 see page 18, paragraph 2 - paragraph 3; claims 1-14; examples 1,2 ---	1-7
X Y	US,A,5 421 816 (LIPKOVKER) 6 June 1995 see abstract see column 15, line 5 - line 18; claims 92-94; figure 19 ---	8 20
	-/--	

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 January 1997	1 0. 01. 97

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 Michels, N |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 323 769 (BOMMANNAN ET AL.) 28 June 1994	8
Y	see abstract see column 3, line 8 - line 16 see column 4, line 32 - column 5, line 2 ---	20
Y	US,A,5 115 805 (BOMMANNAN ET AL.) 26 May 1992	9-15,17, 19-22
A	see abstract see column 1, line 56 - column 3, line 49 see column 7, line 16 - line 20 see column 8, line 17 - line 34; claims 1-3,17 ---	16,18
Y	US,A,5 415 629 (HENLEY) 16 May 1995 see abstract see column 3, line 9 - line 14 see column 4, line 60 - column 5, line 15 see column 7, line 18 - line 33; figure 1 ---	9-14,19, 21,22
Y	EP,A,0 612 525 (SQUIBB BRISTOL MYERS COMPANY) 31 August 1994 see abstract see page 6, line 26 - line 29; claims 1,6,7 ---	20
Y	EP,A,0 368 408 (PROCTER & GAMBLE) 16 May 1990 see abstract see column 1, line 39 - line 48 ---	17
Y	US,A,5 386 837 (STERZER) 7 February 1995 see abstract see column 2, line 16 - line 30; claims 1,2 ---	15
A	WO,A,90 01971 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 8 March 1990 ---	
A,P	US,A,5 445 611 (EPPSTEIN ET AL.) 29 August 1995 -----	

1

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
    because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
    because they relate to parts of the International Application that do not comply with the prescribed requirements to such
    an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
    because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   SEE ANNEX

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
    searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment
    of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
    covers only those claims for which fees were paid, specifically claims Nos.:

    Subject No.1: Claims 1-7
    Subject No.2: Claims 8
    Subject No.3: Claims 9-22

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
    restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

                               ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/210

Subject No. 1: Claims 1-7
        An apparatus applying ultasound to the skin for enhancing molecule
        transport therethrough

Subject No. 2: Claim 8
        A method for collecting analytes through the skin using ultrasound

Subject No. 3: Claims 9-22
        An apparatus applying ultrasound to the skin for enhancing molecule
        transport therethrough in combination with the application of chemical
        enhancers onto the skin and application of mechanical, electric, mag-
        netic, and osmotic force fields

Subject No. 4: Claims 22 bis-24
        A pharmaceutical composition for enhancing molecule transport
        through the skin in combination with applying ultrasound

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8800001	14-01-88	US-A-	4767402	30-08-88
		CA-A-	1324051	09-11-93
		EP-A-	0275284	27-07-88
		JP-T-	1500247	02-02-89
		US-A-	4948587	14-08-90
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		JP-T-	8502424	19-03-96
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		JP-T-	6501855	03-03-94
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		CA-A-	2075624	24-08-91
		EP-A-	0515566	02-12-92
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Form PCT.ISA/210 (patent family annex) (July 1992)

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0368408		CA-A,C	2002298	10-05-90
		JP-A-	2237915	20-09-90
US-A-5386837	07-02-95	NONE		
WO-A-9001971	08-03-90	US-A-	4948587	14-08-90
US-A-5445611	29-08-95	NONE		